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OP 1

Novel treatments for diabetes

0001

Effects of exenatide compared with twice-daily biphasic insulin aspart in patients with type 2 diabetes using metformin and a sulphonylurea

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Background and Aims: In patients with type 2 diabetes (T2D) inadequately treated with oral antidiabetic agents (OADs), studies have shown that exenatide treatment significantly improves glycaemic control with the added benefit of weight reduction. Alternative treatment options include insulin with a formulation containing both intermediate and rapid-acting components. We conducted a 1-y trial to compare the safety and efficacy of exenatide and biphasic insulin aspart 30/70 in patients inadequately treated with metformin (MET) and a sulphonylurea (SU).
Materials and Methods: 66 sites in 13 countries participated in this open-label trial. Intention-to-treat patients with T2D (mean±SD: age 58.7±9.0 y, HbA_{1c} 8.6±1.0%, BMI 30.4±4.1 kg/m^2, body weight 84.4±15.7 kg) were randomised to exenatide (n=253; 5 μg BID for 4 wks, 10 μg BID thereafter) or biphasic insulin aspart (n=248; BID doses individually titrated), adjunctive to pre-existing SU/MET treatment. The primary endpoint in this noninferiority trial was change in HbA_{1c} at Wk 52. The noninferiority margin for the difference between treatments was 0.4%.
Results: The mean dose of biphasic insulin aspart increased from 15.7 U/d (Wk 2) to 24.4 U/d (Wk 52). Both treatments resulted in reductions in HbA_{1c} at endpoint (mean±SEM: exenatide −1.04±0.07%, biphasic insulin aspart −0.89±0.06%; difference −0.15% [95% CI −0.32 to+0.01%]). A greater proportion of exenatide-treated patients achieved target $HbA_{1c} \leq 6.5\%$ (exenatide 18%, biphasic insulin aspart 9%; between-group p=.002) and target $HbA_{1c} \leq 7\%$ (exenatide 32%, biphasic insulin aspart 24%; between-group p=.078). Exenatide-treated patients experienced a steady decline in body weight (−2.5±0.2 kg, p<0.001), while those receiving biphasic insulin aspart gained weight (+2.9±0.2 kg, p<0.001), for a difference between treatments of −5.4 kg (95% CI −5.9 to −5.0 kg, p<0.001). Both treatments reduced fasting serum glucose (exenatide −1.8±0.2 mmol/L, p<0.001; biphasic insulin aspart −1.6±0.2 mmol/L, p<0.001). Between-group analyses revealed greater reductions in 2-h postbreakfast and 2-h postdinner glucose concentrations in the exenatide group, while biphasic insulin aspart predominantly reduced premeal glucose. Nausea (33% incidence, 3.5% withdrawal rate) and vomiting (15% incidence, 1.6% withdrawal rate), mostly mild/moderate intensity, were the most common adverse events reported by exenatide-treated patients. Hypoglycaemia rates were not significantly different between treatment groups (exenatide 4.7±0.7 events/patient–y, biphasic insulin aspart 5.6±0.7 events/patient–y). No severe hypoglycaemia was reported.
Conclusion: Exenatide treatment resulted in overall glycaemic control similar to conventional insulin treatment with biphasic insulin aspart, but without the inconvenience of ongoing titration. Nausea and vomiting were not a significant cause of patient discontinuation from the study. Exenatide reduced fasting glucose, provided better postprandial glucose control, and was associated with weight reduction, making it a potential alternative to biphasic insulin aspart for the treatment of T2D not adequately treated with OADs.
Supported by Eli Lilly and Company and Amylin Pharmaceuticals, Inc.

0002

Safety and effects of a once-weekly, long-acting release formulation of exenatide over 15 weeks in patients with type 2 diabetes

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Background and Aims: Exenatide is an incretin mimetic that improves glycaemic control and reduces body weight when administered twice daily (BID) by subcutaneous (SC) injection in patients with type 2 diabetes treated with metformin and/or a sulphonylurea. This randomized, double-blind, placebo-controlled study assessed the safety, pharmacokinetics and pharmacodynamics of a long-acting release (LAR) formulation of exenatide in 45 patients (40% female, age 53±11 y, BMI 36±6 kg/m^2, WT 106±20 kg, HbA_{1c} 8.5±1.2%, FPG 10±2 mmol/L, diabetes duration 5±4 y, mean±SD) with type 2 diabetes treated with metformin (MET) or diet and exercise (DE).
Materials and Methods: Once-weekly SC doses of either 0.8 or 2.0 mg exenatide LAR, or placebo (PBO) were administered for 15 wks, following a 3-day lead-in period during which patients received either 5 μg exenatide or PBO BID by SC injection.

Results: Dose-related increases in plasma exenatide concentrations were observed, reaching steady-state levels between Wks 6–7. Exenatide LAR 0.8 and 2.0 mg treatment resulted in reductions from baseline in HbA_{1c} (%) of −1.4±0.3 and −1.7±0.3 (mean±SE), and in FPG (mmol/L) of −2.37±0.9 and −2.17±0.5 compared to corresponding increases of+0.4±0.3 and+1.0±0.6 with PBO at Wk 15. With exenatide, the proportion of patients achieving an HbA_{1c} ≤7% was 33% and 86% with 0.8 and 2.0 mg LAR and 0% with PBO. Mean reductions from baseline in body weight (kg) were −0.03±0.7 and −3.81±1.5 in the 0.8 and 2.0 mg exenatide LAR arms versus −0.04±0.7 with PBO. The most frequent adverse event during LAR treatment was mild nausea (LAR 0.8, 2.0 mg: 19%, 27%; PBO: 15%) and no severe hypoglycaemia was observed. No adverse events leading to early withdrawal were reported for the LAR treatment arms.
Conclusion: Exenatide administered weekly as an LAR formulation over 15 wks was well-tolerated, improved glycaemic control, and reduced body weight in a dose-related manner in patients with type 2 diabetes.

0003

Liraglutide treatment, blood pressure and biomarkers of cardiovascular risk in patients with type 2 diabetes: 14 weeks monotherapy study

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Background and Aims: Liraglutide, a once-daily GLP-1 analogue, has been shown to reduce blood glucose and weight in patients with type 2 diabetes. In addition to glucose-lowering effect, the impact of treatment on blood pressure, lipids, adiponectin, leptin, hsCRP, IL-6, TNF-α, PAI-1 and BNP was evaluated.
Materials and Methods: 165 type 2 diabetic patients, drug-naive or on single OAD (washed-out for 4 weeks) were randomised 1:1:1:1 to receive 0.65, 1.25 or 1.90 mg of liraglutide as monotherapy or placebo.
Results: After 14 weeks, significant reduction in SBP vs placebo was observed in liraglutide groups: −7.37 (0.65 mg), −5.21 (1.25 mg) and −7.91 mmHg (1.90 mg) ($p<0.05$ for all). Change in DBP (−1.46, −0.59 and −2.45 mmHg) did not reach statistical significance. Reduction in triglycerides was seen in liraglutide-treated patients when compared to placebo, adjusted differences were −19% (0.65 mg, p=0.03), −15% (1.25 mg, p=0.09) and −22% (1.90 mg, p=0.01). There were no clinically meaningful and consistent differences in total cholesterol, HDL, LDL, ApoB, IL-6, TNF-α, leptin and adiponectin levels between liraglutide groups and placebo (a significantly greater reduction in LDL was seen for placebo vs active dose groups, but this was not confirmed for ApoB where difference was only observed in the 1.25 mg dose group). We saw marked effect of liraglutide on PAI-1 levels of −14% (p=0.29), −29% (p=0.02) and −25% (p=0.05) for 0.65, 1.25 and 1.90 mg liraglutide vs. placebo, respectively. Also, a dose-dependent decrease of BNP levels: −26% (p=0.1), −30% (p=0.05) and −38% (p=0.01) vs. placebo for the respective liraglutide groups. Observed dose-dependent reduction in CRP versus placebo (−3%, −12% and −20%) did not reach statistical significance.
Conclusion: Liraglutide treatment was associated with a decrease in blood pressure, triglycerides, PAI-1 and BNP concentrations, but the observed effects must be confirmed in longer-term studies and need to be further explored.
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0004

Fourteen weeks of liraglutide therapy in subjects with type 2 diabetes significantly improves first phase insulin secretion and maximal beta-cell secretory capacity

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Background and Aims: Liraglutide is a once-daily s.c. GLP-1 analogue under development for the treatment of type 2 diabetes. The data presented here is from a sub-study to a main study including 165 subjects with type 2 diabetes with the aim of assessing beta cell related parameters
Materials and Methods: Subjects were randomized (1:1:1:1) to four treatment arms (placebo, 0.65, 1.25 and 1.90 mg/day of liraglutide). Prior to study drug administration and after 14 weeks subjects underwent both an insulin modified frequently sampled iv glucose tolerance test

(FSIGTT) and a hyperglycaemic clamp (20 mM) combined with and i.v. arginine stimulation.

Results: Out of 39 subjects randomized to the sub-study 28 subjects completed the 14 weeks treatment period. In addition a group of 12 matched healthy subjects (BMI, gender and age) underwent the same experimental procedures (FSIGT and clamp) without being treated with liraglutide. The two highest dosages of liraglutide significantly increased the maximal beta cell secretory capacity compared to placebo, by 6.27 pM (95% CI [2.92; 9.63]) 114% and 7.17 pM (95% CI [3.32; 11.01]) 97% for the 1.25 mg and 1.90 mg doses, respectively ($p<0.05$). The same dosages increased the first phase insulin secretion significantly by 11.0 pM*h (95% CI [6.6; 15.4]) 124% (1.25 mg), and 9.5 pM*h (95% CI [3.5; 15.5]) 107% (1.90 mg) ($p<0.05$). Second phase insulin secretion increased significantly at the 1.25 mg dose level, but did not reach statistical significance at the highest dose level ($p=0.17$ for the 1.9 mg dose group, and $p<0.01$ for the 1.25 mg dose group). No treatment related effect on glucose effectiveness or insulin sensitivity as assessed by the minimal model was seen. For all parameters, except 2nd phase insulin secretion, there was a significantly greater response in healthy subjects in comparison to subjects treated with liraglutide.

Conclusion: Once daily treatment with liraglutide markedly improves beta cell function in subjects with type 2 diabetes.

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0005

Sitagliptin monotherapy improved glycaemic control in patients with type 2 diabetes

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Background and Aims: In response to a meal, glucagon-like peptide-1 and glucose-dependent insulinotropic peptide are released and effect several different processes important for glucose control including stimulating glucose-dependent insulin release, suppressing glucagon release, and delaying gastric emptying. These incretins are, however, rapidly degraded by the enzyme, dipeptidyl peptidase-4 (DPP-4). DPP-4 inhibitors are a novel class of oral antihyperglycemic agents currently in development for the treatment of type 2 diabetes. The aims of this pivotal Phase III study were to examine the efficacy and safety of once-daily sitagliptin, a DPP-4 inhibitor, in patients with inadequately controlled type 2 diabetes (HbA_{1c} ≥7.0% and ≤10.0%).

Methods: This was a Phase III, 24-week, randomized, double-blind, placebo-controlled study. After a drug washout period (for those patients on an antihyperglycemic agent) and a 2-wk single-blind placebo run-in period, 741 patients (ages 18 to 75 years) were randomized to 1 of 3 treatments: placebo, sitagliptin 100 mg q.d., or sitagliptin 200 mg q.d.

Results: For the entire randomized cohort, average baseline HbA_{1c} and FPG were 8.0% and 9.6 mmol/L, respectively. After 24 weeks, sitagliptin 100 mg and 200 mg produced significant ($p<0.001$) placebo-adjusted reductions in HbA_{1c} (−0.79% and −0.94%, respectively) and fasting plasma glucose (FPG: −1.0 and −1.2 mmol/L, respectively). Patients with higher baseline HbA_{1c} (≥9%) had greater reductions in placebo-adjusted HbA_{1c} with sitagliptin (−1.52% for 100 mg and −1.50% for 200 mg) than those with baseline HbA_{1c} <8% (−0.57% and −0.65%) or ≥8% to 8.9% (−0.80% and −1.13%). In a meal tolerance test, sitagliptin 100 mg and 200 mg significantly decreased 2-hr post-meal glucose (PPG) compared with placebo (placebo-adjusted PPG: −2.6 and −3.0 mmol/L, respectively). Post-meal insulin and C-peptide AUC, ratio of insulin AUC to glucose AUC, HOMA-β, and proinsulin/insulin ratio were significantly improved with sitagliptin relative to placebo, suggesting improvements in beta cell function. There was no increased incidence of hypoglycemia or gastrointestinal adverse events with sitagliptin compared with placebo. Minimal reductions from baseline in body weight were observed with sitagliptin 100 mg (−0.2 kg) and 200 mg (−0.1 kg); body weight was significantly ($p<0.01$) reduced with placebo (−1.1 kg) compared to sitagliptin.

Conclusions: In this 24-week study, sitagliptin monotherapy improved glycemic control in the fasting and postprandial states, improved measures of beta-cell function and was well tolerated in patients with type 2 diabetes.

Supported by Merck & Co. Inc.

0006

Efficacy and safety of sitagliptin added to ongoing metformin therapy in type 2 diabetes patients who were inadequately controlled on metformin alone

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Background and Aims: Sitagliptin, a selective dipeptidyl peptidase-4 (DPP-4) inhibitor, is in development for the

treatment of type 2 diabetes. Sitagliptin acts by blocking DPP-4-mediated inactivation of active incretin hormones that are released into the circulation following ingestion of a meal. The incretins regulate blood glucose through a number of glucose-dependent mechanisms, including increased insulin release, suppressed glucagon release, and delayed gastric emptying. This Phase III study assessed the efficacy and safety of sitagliptin added to ongoing metformin therapy in patients with type 2 diabetes who were inadequately controlled (HbA_{1c} ≥7% and ≤10%) on a maximally effective dose of metformin alone.

Material and Methods: After a screening diet/exercise run-in, a metformin dose titration/stabilization period, and a 2-week, single-blind, placebo run-in, 701 patients ages 19 to 78 years (mean HbA_{1c}: 8.0%) receiving ongoing metformin (≥1500 mg/day) were randomized to receive the addition of placebo or sitagliptin 100 mg q.d. in a 1:2 ratio for 24 weeks. Patients not meeting predefined glycemic thresholds were provided rescue therapy (pioglitazone) until the end of the study. The efficacy analysis was based on an all-patients-treated population using an ANCOVA and excluded data obtained after glycemic rescue.

Results: After 24 weeks, the addition of sitagliptin to ongoing metformin therapy led to a significant ($p<0.001$) mean placebo-subtracted reduction from baseline in HbA_{1c} (−0.65%). Treatment with sitagliptin also led to significant ($p<0.001$) mean placebo-subtracted reductions from baseline at Week 24 in fasting plasma glucose (−1.4 mmol/L) and 2-hour post-meal plasma glucose (−2.8 mmol/L). Fasting insulin and C-peptide, post-meal insulin and C-peptide AUCs, post-meal insulin/glucose AUC ratio, and QUICKI, were all significantly increased with sitagliptin relative to placebo. HOMA-β, an index of insulin secretion, was also significantly increased with sitagliptin compared to placebo. In addition, there was a significant decrease in the fasting proinsulin/insulin ratio with sitagliptin relative to placebo, reflecting improved β-cell function. A significantly smaller proportion of patients in the sitagliptin group required glycemic rescue therapy compared to the placebo group. Additionally, the time to initiation of rescue therapy was significantly delayed in the sitagliptin group compared to the placebo group. Sitagliptin was generally well tolerated, with no increased risk of hypoglycemia or gastrointestinal adverse events compared to placebo. The mean change from baseline in body weight at Week 24 was not significantly different between sitagliptin (−0.7 kg) and placebo (−0.6 kg).

Conclusion: In this 24-week study, the addition of sitagliptin 100 mg q.d. to ongoing metformin therapy was well tolerated and provided effective and sustained improvement in HbA_{1c}, FBG, and post-meal glucose, as well as significant improvements in indices of insulin secretion and β-cell function, in patients with type 2 diabetes who had inadequate glycemic control on a maximally effective dose of metformin alone.

Supported by Merck & Co. Inc.

OP 2

Nephropathy – clinical

0007

Mild renal impairment and cardiovascular outcomes in type 2 diabetes: results from the FIELD study

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Background and Aims: Several studies in non-diabetic patients have shown that even mild degrees of renal impairment predict worse cardiovascular outcomes. We examined this hypothesis in a large cohort of patients with type 2 diabetes using estimated glomerular filtration rates.

Materials and Methods: The FIELD study is a randomised double-blind controlled trial of co-micronised fenofibrate 200 mg daily versus placebo in 9795 patients with type 2 diabetes and mild dyslipidaemia over a median follow-up of 5 years in 63 centres in Australia, New Zealand and Finland. Exclusion criteria included a basal serum creatinine of ≥130 μmol/L. We studied only the placebo cohort (n=4900, mean age 62, 63% male) to exclude effects of fenofibrate on plasma creatinine and outcomes. Estimated glomerular filtration rate (eGFR) was calculated by Cockroft–Gault (C–G) and Modification of Diet in Renal Disease method (MDRD)

Results: Total cardiovascular (CV) event rate were calculated according to estimated GFR in bands of <60, 60–90 and >=90 ml/min/1.73 m^2 or ml/min respectively. Patients with C–G eGFR <60 showed a CV event rate 1.98 fold that of those with eGFR >=90 while those with eGFR of 60–90 showed a 1.33 fold excess ($p<0.0001$ for both). Parallel analyses with MDRD eGFR gave corresponding hazard ratios of 3.57 and 1.75 respectively ($p<0.0001$ for both). These effects persisted after correction for systolic and diastolic blood pressure (BP) or for mean BP: ratios 1.78 and 1.27 for C–G; 3.23 and 1.75 for MDRD (all $p<0.0001$). The CV event hazard ratios were greater than those seen for macroalbuminuria and microalbuminuria compared with normoalbumninuria, and identified a small group of patients with an extremelyhigh rate of one or more CV events over 5 years.

Conclusion: We conclude that relatively minor degrees of renal impairment are associated with a worse cardiovascular prognosis and that eGFR, especially by the MDRD method, may be a useful marker for this. It may be simpler, more

consistent and less modified by treatment than BP or degree of proteinuria, and thus allow more accurate risk assessment and improved targeting of cardioprotective therapy.

0008

Serum prorenin, renin genotype and glycated haemoglobin are predictors of persistent microalbuminuria in type 1 diabetes

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Background and Aims: The renin angiotensin aldosterone system (RAAS) is implicated in the development of diabetic nephropathy (DN). The most striking abnormality is an increase in prorenin, the enzymatically inactive precursor of renin. Previously we observed in two separate cross-sectional cohort studies with varying duration of diabetes that increased prorenin and HbA_{1c} predict the development of microalbuminuria, and that renin genotype for a biallelic polymorphism may weakly influence risk of DN. The aim of the present study is to study prorenin, HbA_{1c} and renin genotype in a prospective follow-up study of an inception cohort of 277 patients with newly diagnosed type 1 diabetes.
Materials and Methods: An inception cohort of 277 patients with newly diagnosed type 1 diabetes, consecutively admitted to the Steno Diabetes Center between 1 September 1979 and 31 August 1984. Development of persistent microalbuminuria was the main outcome measure. Serum prorenin, HbA_{1c} and renin genotype were evaluated as predictors for this outcome.
Results: During a median follow-up of 18.0 years (range 1.0–21.5 years), 79 of 277 patients developed persistent microalbuminuria. At baseline sex, median albumin excretion rate, and systolic and diastolic blood pressure differed between subjects that eventually developed microalbuminuria and those that did not (respectively 70 vs. 55% male, p=0.02, 11 vs. 8 mg/24 h, p=0.002, systolic blood pressure 128 vs. 122 mm Hg and diastolic blood pressure 80 vs. 76 mm Hg, p=0.004 for both). Univariate Cox regression analysis with persistent microalbuminuria as dependent variable showed prorenin, HbA_{1c}, mean arterial blood pressure (MABP) (all as time-dependent variables), and sex to be significant covariates. Renin genotype data were available for 223 subjects. In multivariate Cox regression analysis MABP and sex lost significance. In the final model HbA_{1c}, serum prorenin and renin genotype (collapsed to absence or presence of one or two copies of the most frequent allele) were significant covariates (p=0.001, 0.008 and 0.026 respectively). An even stronger covariate than absolute prorenin level was prorenin increase from baseline prorenin (measured in the 1st or 2nd year of diabetes), β=3.004, p=0.001). No interaction was observed between prorenin level and renin genotype. A prognostic index, indicating risk of microalbuminuria, can be calculated from the formula: 1.11*renin genotype (0 or 1)+2.344 * log [prorenin]+0.435 *HbA_{1c}.
Conclusion: An increase in serum prorenin and HbA_{1c} predicts persistent microalbuminuria, the herald of diabetic nephropathy. Renin genotype appears to modify risk as well, but this does not occur through increased prorenin level. From a clinical point of view our findings allow identification of normoalbuminuric subjects at high risk and should lead to a study that assesses the benefits of starting treatment (e.g. blockade of the RAAS) in such patients.
Supported by the Danish Diabetes Association

0009

Plasma α-defensin is associated with diabetic nephropathy and mortality in type 1 diabetic patients

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Background and Aims: α-defensins are antimicrobial peptides of the innate immune system. They are secreted by neutrophils during phagocytosis. In addition, α-defensins are suggested to be involved in atherosclerosis by stimulating binding of lipoproteins and LDL cholesterol to endothelial cells in the blood vessels. By binding to tissue-plasminogen activator, α-defensins inhibit fibrinolysis, causing thrombotic occlusions. Finally, α-defensins may participate in angiogenesis through binding to integrin molecules. To elucidate a potential role of α-defensin in micro- and macrovascular complications, we investigated the association between plasma levels of α-defensin and the presence of diabetic nephropathy as well as cardiovascular disease (CVD) in type 1 diabetic patients.
Materials and Methods: A total of 391 patients with long lasting type 1 diabetes were recruited through the outpatient clinic at Steno Diabetes Center, Gentofte. Approximately half of the study population (n=199) had diabetic nephropathy, the rest (n=192) had normoalbuminuria. After an overnight fast, venous blood was drawn from an

antecubital vein and plasma stored at −80°C. Occurrence of CVD and ischemic heart failure were evaluated. Blood pressure, se–creatinine, BMI, HbA_{1C}, cholesterol levels and other clinical parameters were measured. To study the association between plasma α-defensin, the development of vascular complications and mortality, we prospectively followed the study cohort for more than 10 years. Plasma α-defensin was measured using a validated in-house radioimmunoassay (RIA).

Results: The median plasma α-defensin concentration at baseline was 258 μg/L (IQR 205–321 μg/L). Patients with nephropathy had significantly higher levels of plasma α-defensin compared to those with normoalbuminuria: 305 μg/L (IQR 182–263) vs. 223 μg/L (IQR 205–321), $P<0.0001$. This difference remained significant after adjustment for diabetes duration, HbA1c, BMI, blood pressure, UAE and GFR; nephropathy vs. normoalbuminuria: 302 μg/L (95% CI 287–317) vs. 250 μg/L (95% CI 235–266), $P<0.0001$.

During follow-up 76 patients died, hereof 32 from CVD. Patients who died had significantly higher levels of α-defensin than survivors: 335 μg/L (IQR 269–394) vs. 245 μg/L (IQR 200–301), $P<0.0001$. From ROC curve analysis, an α-defensin level of 310 μg/L was found to be the best predictive cut-off level (sensitivity 0.61, specificity 0.81, area under the ROC curve 0.74, $P<0.0001$). All-cause mortality in patients with plasma α-defensin levels >310 μg/L was 43% (46 deaths among 107 patients) vs. 11% (30/282) among patients with α-defensin <310 μg/L, hazard ratio 4.9 (95% CI 3.1–7.7), $P<0.0001$. Of note, a plasma α-defensin level >310 μg/L remained an independent predictor of mortality, even after adjustment for nephropathy status at baseline and for the possible confounding effects of age, sex, $HgbA_{1c}$, plasma creatinine, BMI, total cholesterol and systolic blood pressure (hazard ratio 2.0, 95% CI 1.1–3.6, $P<0.05$).

Conclusion: Our data indicate that plasma α-defensin may be related to the development of diabetic nephropathy. Further, our findings suggest that measurements of plasma levels of α-defensin may be used as a prognostic factor for nephropathy and mortality in type 1 diabetic patients.

Supported by the Danish National Board of Health

0010

Polymorphisms in the adiponectin gene are associated with the incidence of renal event in type 2 diabetic subjects with a 4-year follow-up

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Background and Aims: Adiponectin concentration is decreased in Type 2 diabetic (T2D) subjects, and subjects with coronary artery disease. However the concentration of this adipokine is increased in subjects with renal disease. Our objective was to evaluate the association between three adiponectin gene polymorphisms (SNP), −11391 G>A,+45 T>G and+276 G>T, and the prospective risk of renal event in a cohort of T2D subjects: the Diabhycar study

Materials and Methods: Participants were 3,123 French T2D with high vascular risk from the DIABHYCAR Study (non-insulin-dependent DIABetes HYpertension microalbuminuria or proteinuria, CArdiovascular events, and Ramipril). The participants were older than 50 years and had urinary albumin excretion ≥20 mg/l. The renal events incident cases (n=75) were defined as creatininemia doubling and/or end stage renal disease. The subjects were genotyped by using the TaqMan® allelic discrimination and the Molecular Beacons®. We achieved survival analysis with multiple adjustment by using the Cox regression analysis.

Results: The −11391 AA genotype was associated with higher baseline urinary albumin excretion (194.0 mg/l in AA vs 97.4 mg/l in G carriers, p=0.002). In this cohort, the risk of incident renal event was higher in −11391 A carriers (HR 1.89 [1.11–3.20], p=0.019, adjusted for baseline values of HbA1c, HDL-cholesterol, log [creatinine], log [CRP] and log [urinary albumin excretion]), and higher in +45 G carriers (HR 2.18 [1.31–3.60], p=0.003). The highest risk was found for the combination of both alleles (+45G/−11391A vs+45T/−11391G: OR 3.61) indicating that the effects of the two polymorphisms were cumulative. Furthermore, the +45 T>G and −11391 G>A SNPs were associated with the 4-year increase in creatinine levels (respectively p=0.001 and p=0.043). The+276 G>T SNP was associated neither with the incidence of renal events nor with variations in serum creatinine levels.

Conclusion: Our results show that adiponectin genetic polymorphisms are predictive for the incidence of renal complications in type 2 diabetic patients. Our data support a causal role of the adiponectin in the incidence of renal disease.

0011

Pharmacogenetic association of the ACE/ID polymorphism on renal outcome and death in relation to losartan treatment in patients with type 2 diabetes and nephropathy

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Background and Aims: The RENAAL study showed that losartan treatment significantly reduced renal outcomes in patients with type 2 diabetes and nephropathy. We investigated the whether an insertion (I)/deletion (D) polymorphism in the angiotensin-I-converting enzyme (ACE) gene predicts renal outcomes and death and influences with the effect of losartan in the RENAAL study.
Materials and Methods: A total of 1435 (95%) out of 1513 patients enrolled in this randomized, double blind study comparing losartan with placebo on top of conventional blood pressure lowering therapy were available for this investigation. The primary outcome was the composite of doubling of baseline creatinine concentration, end stage renal disease (ESRD) or death. This endpoint was analyzed using a stratified COX regression model with terms including treatment, geographic region, ACE/ID genotype and treatment-by-genotype interaction.
Results: The composite endpoint was lower in II vs. ID and DD, 17.5% and 38.1%, P=0.029. The individual components of this endpoint showed the same trend. The adjusted risk reduction for losartan versus placebo for the composite endpoints was 5.8% (95% CI −23.3, 28.0), 17.6% (3.8, 29.4) and 27.9% (7.0, 44.1) for II, ID and DD respectively. A significant interaction between losartan treatment and ACE/ID genotypes was demonstrated for the risk reduction for ESRD of 3.1%, 30.5% and 50.4% for II, ID and DD respectively (P=0.032). A similar trend was demonstrated for the other endpoints.
Conclusion: Proteinuric Type 2 diabetic patients with the D allele of the ACE/ID polymorphism have an unfavorable renal prognosis which can be mitigated and even improved by losartan.
Supported by Merck

0012

Improved survival of patients with diabetes and end-stage renal disease in Denmark

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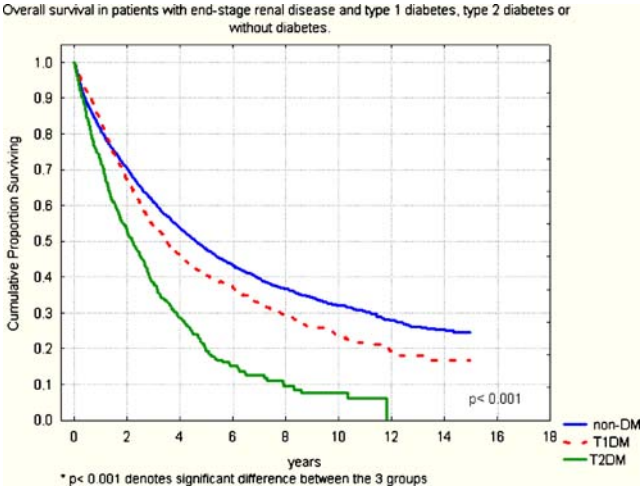
Background and Aim: Treatment in patients with diabetic nephropathy has improved during the last 15 years. Whether life expectancy of diabetic patients with end-stage renal disease (ESRD) has improved is unknown. The aim of our study was to investigate the survival of diabetic patients with ESRD in Denmark in the time period from 1990–2004, and to evaluate the effect of type of diabetes, transplantation, age and calendar time.
Materials and Methods: Data were obtained from The Danish National Registry; Report on Dialysis and Transplantation, where all patients initiating active treatment for ESRD has been registered and followed up since 1990. ESRD is defined as need of chronic dialysis or renal transplantation. The patients were stratified into 3 groups according to the 5-year period during which renal replacement therapy was initialized. Log-rank test was used to compare survival rates and cox-regression to estimate proportional hazards.
Results: A total of 8618 patients (1123 (13%) with type 1 diabetes (T1DM), 719 (8%) with type 2 diabetes (T2DM) and 6776 (79%) without diabetes (non-DM)) initiated renal replacement therapy during the study period. Mean age (years)(SD) was: T1DM: 50(14), T2DM: 66(9), non-DM: 58(17). The overall patient survival rate is shown in figure 1. The survival of T1DM patients has approached that of non-DM patients and was superior to patients with T2DM. Receiving a renal transplantation was the most important predictor of survival with 80% lower mortality risk (hazard ratio (HR)=0.20 95% CI: 0.18–0.22) compared to patients on dialysis. Patients with DM (T1 and T2) had a 56% increased mortality risk (HR=1.56 CI 1.46–1.68) compared to non-DM patients, whereas the HR for age was 1.4 (CI: 1.36–1.43) per decade of increasing age. Survival improved during the study period with a 21% lower mortality risk (HR=0.79 CI: 0.73–86) in patients with DM (T1 and T2) and a 15% lower mortality risk in non-DM patients (HR=0.87 CI: 0.83–0.91) per 5 calendar years. The percentage of patients who received a transplantation was: T1DM: 27%, T2DM: 5% and non-DM: 26%. The survival of transplanted patients with DM (T1 and T2) compared to non-DM patients at one year was: 95% vs. 93%, 5 years:

80% vs. 85% and 10 years: 52% vs. 71%. There was no difference in graft survival between the two groups.

Conclusion: The survival of diabetic patients with ESRD has markedly improved during the past 15 years in Denmark. The overall survival of T1DM patients has approached that of non-DM patients and the percentage of patients who were receiving a renal transplantation was similar among T1DM and non-DM patients. The 5-year survival after renal transplantation in DM (T1 and T2) patients was 80% and comparable to the survival in patients without diabetes.



Supported by Nyreforeningen, Diabetesforeningen

OP 3

Immunopathogenesis of type 1 diabetes – from mice to men

0013

Peripherin is the major neuroendocrine autoantigen targeted by islet-infiltrating B-lymphocytes in NOD mice

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Background and Aims: B-lymphocytes migrate to the pancreatic islets during T1D development. Our current appreciation of the antigenic repertoire of autoreactive B-cells in T1D almost exclusively stems from studies of the antigenic specificity of circulating islet-reactive autoantibodies and from analyses of the antigenic specificity of peripheral B-cell hybridomas from human blood or rodent spleen. In order to characterize the antigenic specificity of islet-infiltrating B-lymphocytes during the progression of the disease, we generated hybridoma cell lines of these infiltrating B-cells rom NOD and (NODxNOR)F1 mice. We found that a prevalent number of hybridomas (55,8%) produced MoAbs restricted to peripheral nervous system (PNS) elements. The aim of the present study has been to characterize the molecular nature recognized by these MoAbs.

Materials and Methods: On neuroblastoma and insulinoma cell lines, MoAbs anti-PNS displayed a reticular intracellular staining. Then we pursued a fractioned protein extraction protocol from these cell lines, western blot immunodetection on protein extracts, isolation and trypsin digestion of the spot recognized by MoAbs, and subsequent mass-spectrometry analysis. An intermediate filament network-defective cell line was transfected with plasmids encoding the peripherin isoform Per56, Per58 or Per61. Specificity of MoAbs for every isoform was assessed by Western blot analysis.

Results: Interestingly, our studies revealed that peripherin was the autoantigen recognized by all the MoAbs (21/21). Peripherin is an intermediate filament protein expressed in PNS as well as in pancreatic beta cells. Three peripherin isoforms generated by alternative splicing have been described. Our studies indicate that all MoAbs target Per58 and Per 61 isoforms, whereas only one (1/21) binds Per56 isoform as well. Since Per56 isoform differs from Per58 and Per61 in the C-terminal aminoacidic sequence, the results strongly suggest that the epitope recognized by most of MoAbs is found at the carboxiterminal end of the Per58 and Per 61 isoforms.

Conclusions: Peripherin is the major neuroendocrine autoantigen targeted by islet-infiltrating B-lymphocytes. The dominant(s) epitope(s) of peripherin recognized by islet-infiltrating B-lymphocytes maps at the carboxi-terminal region of the protein.

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0014

A unique CD204^{+} subpopulation of dendritic cells in NOD mice

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Background and Aims: Dendritic cells (DC) are professional antigen presenting cells, and central to the immune system. DC display defective function and phenotype in human type 1 diabetes, as well as in NOD mice and BB rats,

and possibly play crucial roles in the pathogenesis of this disease. To characterise molecular changes in $CD11c^+$ bone marrow (BM)-derived DC from NOD, we recently compared transcript profiles of these cells with those from normal NON mice, and then found that BMDC from NOD express significantly higher level of CD204 (macrophage scavenger receptor type A) than those from NON. Interestingly in monkeys, CD204 on DC reportedly mediates a unique capacity to capture antigens from live cells, a process referred to as nibbling. Given that CD204 intervenes in this process irrespective of race, strong expression of CD204 on DC from NOD possibly leads to active antigen uptake from autologous cells, and then to the predisposition to autoimmune diseases. Focusing on this point, we here investigated the phenotype and function of $CD204^+$ BM and splenic DC from NOD, and the role of this molecule in nibbling.

Materials and Methods: Bone marrow cells from 6-week-old female mice were cultivated in the presence of GM-CSF and IL-4 over 6 days. $CD11c^+$ DC were sorted by magnetic beads-conjugated anti-CD11c antibodies (MACS™). BM and splenic DC were phenotyped by a flow cytometer. Syngeneic mixed lymphocyte reaction was performed co-culturing $CD204^+$ or $CD204^-$ DC sorted with FACSAria™ and syngeneic $CD4^+$ T cells, and then measured with the MTT assay. $CD11c^+$ BMDC were dual labeled with the fluorescent dyes DiO and DiD, and nibbling was evaluated by monitoring the trafficking of plasma membrane between labeled and unlabeled cells.

Results: BMDC from 6-week-old female NOD displayed 7–8 times higher mean fluorescence intensity of CD204, than normal strains (NOD 65.7±3.4, NON 8.2±0.34, C3H/HeN 8.0±0.55, Balb/c 9.0±0.49, $p<0.05$, Mann–Whitney U test). Among splenocytes, the proportion of $CD204^+$ $CD11c^+$ DC from 6-week-old female NOD was significantly higher than normal strains (NOD 0.65±0.09%, NON 0.10±0.02%, C3H/HeN 0.10±0.02%, Balb/c 0.04±0.03%, $p<0.05$). The phenotype of $CD204^+$ $CD11c^+$ BM and splenic DC was $CD4^{low}CD8^-CD11b^{low}CD16/32^+CD19^{low}CD80^{high}CD86^{high}$MHC class I^+ class $II^+F4/80^+Gr\text{-}1^{low}$. $CD204^+$ $CD11c^+$DC showed equivalent capacity to stimulate syngeneic $CD4^+$ T cells to $CD204^-$ $CD11c^+$DC (Splenic DC, OD 0.10±0.03 vs 0.10±0.10; BMDC, OD 0.26±0.02 vs 0.25±0.01, stimulator: responder=1:100, NS). Unlabeled BMDC from NOD acquired fluorescently dual labeled membrane lipid from other cells more rapidly than those from NON (Figure). The trafficking of plasma membrane was blocked with polyG, one of CD204 ligands.

Conclusion: We propose that CD204 highly expressed in a unique subpopulation of DC from NOD possibly leads to active uptake of self-antigens, and then to the predisposition to autoimmune diseases in NOD. The effect by genetic disruption of CD204 on NOD is now under investigation.

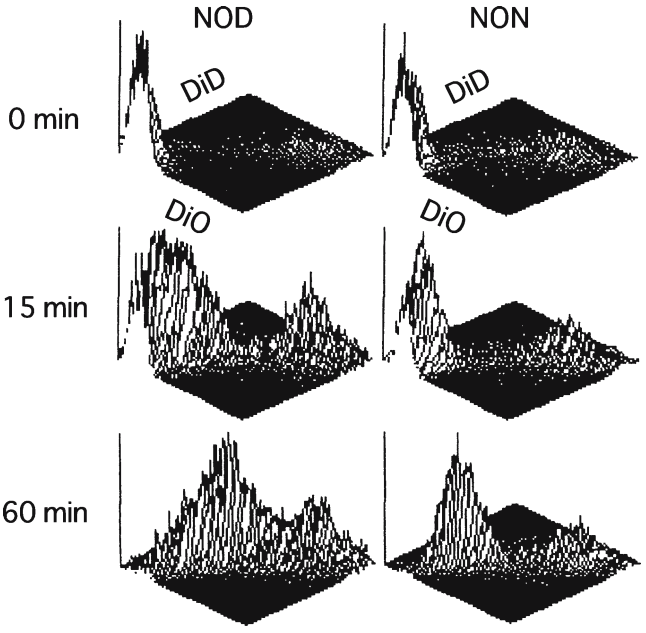

Figure Flow cytometric analysis of plasma membrane trafficking between BMDC dual labeled with DiO and DiD and those unlabeled.

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0015

Unaltered development of insulitis and diabetes in vitamin D receptor KO NOD mice

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Background and Aims: 1,25-dihydroxyvitamin D_3 or active vitamin D prevents type 1 diabetes in animal models, modulates dendritic cell differentiation and induces regulatory T cells. On the other hand, vitamin D deficiency increases the incidence of type 1 diabetes in genetically-predisposed individuals and results in impaired maturation of bone marrow-derived macrophages. This steroid hormone, which is also produced in macrophages and dendritic cells, acts through specific binding to a member of the nuclear receptor family, the vitamin D receptor (VDR). The aim of the present study was to evaluate the effect of eliminating VDR in non-obese diabetic (NOD) mice on disease pathogenesis.

Materials and Methods: This was done by introducing the VDR-null mutation onto the NOD genetic background, by backcrossing $VDR^{-/-}$ mice (C57BL/6) into the NOD strain for 14 generations.
Results: VDR-deficient NOD mice (on regular diet containing 1% calcium, 1.25% phosphorus and 2500 IU/kg vitamin D) had typical features of vitamin D-dependent rickets type II, including hypocalcaemia, osteomalacia, and alopecia. $VDR^{-/-}$ NOD mice had growth retardation as reflected by decreased body weight during development. Despite these characteristics, lack of VDR did not affect the development of islet cells and the general morphology of islets in NOD mice as compared to wild-type mice, in contrast to previous observations in NOD mice lacking the ligand, vitamin D. Moreover, VDR-deficient NOD mice developed insulitis similar to VDR-sufficient NOD mice. Development of diabetes by the age of 250 days in the N10 generation was identical between VDR-deficient and -sufficient NOD mice, in final outcome (in females 70% vs 80%, NS; in males 40% vs 40%, NS) as well as in kinetics (onset of diabetes; in females 142±18 vs 139±13 days, NS; in males 163±7 vs 166±10 days, NS). The cumulative diabetes incidence in mutant females remained unchanged after increasing the number of backcrosses to N14 (69% vs 70% in wild type females), as was the case in males (30% vs 38% in wild type males, NS). As expected, no major immune defects between $VDR^{-/-}$ and $VDR^{+/+}$ NOD mice were observed at 100 days of age. Lymphocyte subset composition in different immune organs was normal, apart from a profound decrease in immature $CD4^{-}CD8^{-}$ and regulatory $CD4^{+}CD25^{+}$ T cells. Splenocyte proliferation was normal after aCD3 stimulation, but a lower response to alloantigens was observed in a mixed lymphocyte reaction. No differences in phagocytic capacity of macrophages and neutrophils were observed between the groups.
Conclusion: These results suggest that VDR is not critically involved in islet formation and that VDR expression is not an absolute requirement for development of autoimmune diabetes in the NOD mouse.

0016

Characterisation of the T-cell repertoire in the LEW.1AR1-iddm rat

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Background and Aims: The LEW.1AR1-*iddm* rat is an animal model of human Type 1 diabetes mellitus (T1DM) which arose through a spontaneous mutation in the diabetes resistant inbred strain LEW.1AR1. Recently, flow cytometry of peripheral blood lymphocytes (PBL) indicated that the T-cell content of LEW.1AR1-*iddm* rats is altered as compared to the background strain LEW.1AR1. The aim of this study was to characterise the lymphocyte repertoire within the PBLs of the diabetes susceptible LEW.1AR1-*iddm* strain and its diabetes resistant control strain LEW.1AR1 during the first twelve weeks of life.
Materials and Methods: Blood of the two congenic LEW strains, LEW.1AR1-*iddm* (diabetic) and LEW.1AR1 (diabetes resistent) was taken every five days between 30–120 days of age. PBLs were differentiated by flow cytometry using a set of monoclonal antibodies (OX-38 FITC, OX-8 FITC, OX-8 PE, G4.18 PE, R73 FITC, OX-33 FITC, NKR 10/78 FITC, all BD; ED1 FITC, Serotec).
Results: The LEW.1AR1 strain was characterised by an age-dependent composition of lymphocyte subpopulations within the PBLs. In contrast, the course of the lymphocyte composition within the PBLs of the LEW.1AR1-*iddm* rats during the first twelve weeks of life was almost unique for each single animal. The alterations of the T-cell subpopulations in the course of life of the LEW.1AR1-*iddm* rats lead to a strong variation of the $CD4^{+}/CD8^{+}$ T-cell ratio, varying between 0.9 and 2.2 with a mean value of 1.52 (±0,06). The $CD4^{+}/CD8^{+}$ T-cell ratio in PBLs of the background strain LEW.1AR1 was much more stable with a mean values of 2.1 (±0,02).
Conclusion: The mutation of the LEW.1AR1-*iddm* rat has an effect on the lymphocyte composition of the PBLs at variance from the coisogenic background strain LEW.1AR1. Moreover, it can be postulated that these alterations lead to T1DM development in 60% of the LEW.1AR1-*iddm* colony. Interestingly, recent investigations in diabetic patients also indicate that alterations in the lymphocyte repertoire may play a role in the pathogenesis of human T1DM. Perspectively, the identification of the functional role of the variant T-cell content may help to understand the mechanisms of autoimmunity in T1DM.

0017

The 70 kDa heat shock protein DnaK targets the same B-chain epitope of human insulin as autoimmune T-cells

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Background and Aims: Autologous insulin represents a dominant antigen in the development of immune reactivity against pancreatic beta cells. The hormone is a target of autoantibodies and of cell-mediated immune processes including insulin-reactive T-cells, which emerge in the prediabetic phase in type 1 diabetes and in animal models of the human disease. Recent studies have shown that heat shock proteins (Hsp) have a high capacity to stimulate the proinflammatory activity of innate immune cells, such as macrophages. In addition, due to their chaperone activity, Hsp can induce peptide-specific immune responses by efficient transfer of peptides to MHC molecules for antigen presentation. In the present study we investigated the potential interaction of insulin with Hsp70 as a critical initial event in the selection of beta cell-specific (auto-) antigenic peptides.
Materials and Methods: The specificity of Hsp-insulin interactions was studied by analysing the binding of DnaK, an Hsp70 homolog of *Escherichia coli*, to preproinsulin-derived 13-mer peptides and to (pro-)insulin variants.
Results: First, we studied the binding of DnaK to immobilized 13-mer peptides spanning the entire 110 amino acid (aa) sequence of preproinsulin. Four clusters of the preproinsulin peptides exhibited strongly increased DnaK retention in a range similar to those of control peptides with well-documented high affinities to DnaK. Each of the four functional preproinsulin domains contained one DnaK binding region: the signal peptide (aa S7–23), the A-chain (aa A6–21), the B-chain (aa B11–25) and the C-peptide (aa C15–31). The binding strength of DnaK to the immobilized peptides was assessed by determining the kinetics of DnaK release from the peptides during fractionated electro-elution. In this approach peptide B11–23 exhibited the strongest binding to DnaK of all 13-mer peptides within the proinsulin sequence. In addition, strong binding of DnaK to the signal peptide was observed. The apparent dissociation equilibrium constants (Kd) of the complexes of DnaK and selected 13-mer insulin peptides, proinsulin and insulin were determined by a competition binding assay using a fluorescence-labeled peptide with high affinity to DnaK. The B-chain region B11–23 exhibited high affinity for ADP-liganded DnaK, the substrate sequestering form of the chaperone (Ki 2.2±0.4 μM). This region had previously been identified as dominant T-cell epitope and includes the hydrophobic core motif Lys–Tyr–Lys, which also represents a binding motif for MHC. However, a Tyr to Ala exchange did not affect the affinity of the peptide to DnaK (Ki 1.8±0.3 μM). The physiologically active insulin heterodimer shows extremely low DnaK affinity (Ki 67.8±21.0 μM) while an only weakly aggregating proinsulin variant exhibited substantial binding (Ki 11.3±7.8 μM).
Conclusion: Our observations demonstrate a high affinity of Hsp70 to distinct proinsulin-peptides with a preference for the B-chain peptide B11–23. We conclude that the initial preselection and chaperoning of sets of potentially (auto-)antigenic peptides by Hsp might link innate with adaptive immunity and thus play a central role in the induction of immune reactivity directed against autologous antigens such as insulin.

0018

Reduced expression of regulatory T-cell associated transcription factor Foxp3 in cord blood T lymphocytes from newborn infants at genetic risk of type 1 diabetes

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Background and Aims: In type 1 diabetes the function of the regulatory T cells has been reported to be deficient. The aim of this study was to characterize the in vitro expression of regulatory T-cell related transcription factor Foxp3 in cord blood (CB) lymphocytes from newborn infants carrying the HLA risk haplotypes for type 1 diabetes.
Materials and Methods: CB EDTA samples were randomly received from newborn infants taking part in the Trial to Reduce IDDM in Genetically at Risk (TRIGR) project with a first-degree relative (mother, father, or full-sibling) with type 1 diabetes or the Finnish Dietary Intervention Trial for Prevention of type 1 diabetes (FINDIA) project with no affected family member. We included 16 infants carrying DR4–DQ8 (DQB1*0302) haplotype, 12 infants with DR3–DQ2 (HLA DQA1*0501–DQB1*02) haplotype, and 36 newborn infants who did not have either DR4–DQ8 or DR3–DQ2 haplotype. CB mononuclear cells were stimulated in type 1 (IL-12, anti-IL-4), or type 2 cytokine (IL-4, anti-IL-12) environment for 6 days. Expression of transcription factor Foxp3 was determined by real time quantitative RT–PCR.
Results: After culture of CB mononuclear cells in type 2 cytokine environment the expression of Foxp3 differed between the groups with various HLA genotypes (p=0.020, Kruskal–Wallis test). The relative level of Foxp3 mRNA was lower in newborn infants carrying DR4–DQ8 haplotype compared to subjects with DR3–DQ2 haplotype (median 11.1 vs 18.50; p=0.011, Mann–Whitney U-test) and newborn infants without DR4–DQ8 or DR3–DQ2

haplotypes (median 11.1 vs 18.10; p=0.013). No difference was seen between groups grown in type 1 cytokine environment.

Conclusion: The up-regulation of Foxp3 is a prerequisite for the function of regulatory T-cells, and the poor activation of Foxp3 in the infants with HLA DR4–DQ8 may explain their susceptibility to autoimmunity later in their life.

OP 4

TCF7L2 – a novel gene for type 2 diabetes

0019

Large scale case-control and family-based analyses of *TCF7L2* variants in > 6000 UK subjects demonstrates an almost two-fold difference in relative risk between homozygote classes

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Background and Aims: Evidence has recently emerged relating common variation in the gene *TCF7L2* (encoding transcription factor 7 like 2) to type 2 diabetes susceptibility in subjects of Northern European origin. The generalizability of these findings and the mechanisms involved are, as yet, unknown.

Materials and Methods: We typed four *TCF7L2* SNPs (the two most associated in the previous study [rs12255372 & rs7903146] plus the two best correlated proxies available from Phase II Hapmap [rs4506565 & rs12243326]), and performed a case-control analysis in 2158 UK T2D subjects and 2574 controls, supplemented with family-based association analysis in 388 parent-offspring trios. Genotyping was performed using a fluorescence-based competitive allele-specific PCR method (KASPar) with overall call rates for each SNP exceeding 95%. All SNPs performed well against stringent quality control criteria. There was no evidence of departure from Hardy–Weinberg equilibrium (all P>0.05 in controls), two Mendelian inconsistencies in 963 families and a discrepancy rate on duplicate genotyping of 2/2416 (ie 0.04% error).

Results: Case-control analysis demonstrated powerful association with T2D at all four SNPs, with the strongest effect at rs27903146 (allele-wise Relative Risk 1.36 95% CI 1.24–1.48) ($P=1.3\times10^{-11}$). Data were consistent with a multiplicative model. Compared to baseline, the genotype relative risk of diabetes in individuals carrying two copies of the risk allele was 1.90 (95% CI 1.54–2.33) ($P=3.6\times10^{-10}$). Independent evidence for association at all loci was provided by the family-based analyses (eg. TDT at rs4506565 showed 62% transmission, $P=7\times10^{-5}$) with no parent of origin effects evident. *TCF7L2* genotypes associated with T2D were more frequent in cases selected for positive family history and early onset (rs4506565 $P=0.02$), and the population attributable risk (based on the least selected cases) was estimated at 16%. No aetiological mutations were detected when all exons were re-sequenced in 48 subjects with permanent early-onset diabetes. The global evidence for association, derived by combining case-control and family-based analyses, exceeded genome-wide significance ($P=4.4\times10^{-14}$).

Conclusion: Our results provide overwhelming support and replication of the previously published association between *TCF7L2* variation and type 2 diabetes demonstrated in Icelandic, Danish and US cohorts. Indeed, *TCF7L2* variation confers the greatest T2D-susceptibility effect yet demonstrated. The effect size and allele frequency of this variant augurs well for ongoing efforts to identify additional diabetes susceptibility genes through genome wide approaches.

0020

TCF7L2 gene strongly associates with type 2 diabetes, with lower age of onset and BMI in the French population, and its adipose expression is impaired in diabetes

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Background and Aims: Recently, non-coding variants in TCF7L2, a transcription factor in the Wnt signaling pathway, were strongly associated with increased risk of type 2 diabetes (T2D) in samples from Iceland, Denmark and the US. We analyzed the two most associated variants in the French population.
Materials and Methods: Case-control study was carried out with 2,421 obese and lean T2D patients and in 926 control individuals (BMI <27 kg/m^2 and fasting glycemia <5.6 mmol/l) from a French population. High-throughput genotyping of rs7903146 and rs12255372 variants was performed by Taqman (Applied Biosystem). Chi^2 test and P values were empirically computed with the CLUMP program for analyses. A multivariate linear regression model taking into account age and sex was performed for BMI parameters. The same analysis was done with BMI adjustment for diabetes onset parameters. Affymetrix microarrays and MTC panels were used to assess the TCF7L2 expression in different tissues.
Results: We confirmed the previously-showed association of TCF7L2 variants (rs7903146 and rs12255372) with risk of T2D in French population (respectively OR=1.69, P=1.7 10–19 and OR=1.64, P=8.2 10–17). Due to strong linkage disequilibrium between the two variants (delta=0.86), we decided to only analyse the most associated with T2D (rs7903146). We found a strong OR increase (OR=1.93, P=1.3 10–24) in T2D non-obese subgroup (BMI <30 kg/m^2, 926 control individuals, 1265 T2D patients). Contrary to the previously published data, we found that the at-risk allele is associated with lower BMI (P=2.8 10–4). In addition, there was a 2.4 year decrease of the age at diabetes onset with the at-risk allele (P=3.7 10–5). In human tissues, TCF7L2 expression was found to be quite ubiquitous although poorly expressed in the skeletal muscle. Its expression pattern looks like following NGN3 expression during the early steps of rat foetal β-cells differentiation. In subcutaneous and omental adipose tissues, the TCF7L2 expression is impaired in glucose-intolerant and diabetic obese subjects.
Conclusion: In French population, we found a very strong association between two previously studied TCF7L2 variants and T2D, especially in non-obese subjects. These variants are also associated with early diabetes onset and a lower BMI. In humans, TCF7L2 expression is ubiquitous and is impaired in the diabetes state. TCF7L2 is particularly expressed at early steps of β-cells differentiation, suggesting a physiological role in several key organs for glucose homeostasis.

0021

Common variants in the *TCF7L2* and *PPARG* genes predict future type 2 diabetes in 7,000 persons followed for 22 years from the Malmö preventive project (MPP)
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Background and Aims: Type 2 diabetes (T2D) is considered a complex polygenic disorder where common genetic variants interact with environmental factors to unmask the disease. Common variants in a number of genes have been associated with type 2 diabetes in cross-sectional studies. The question whether this information could be used to identify individuals at risk of developing future diabetes has been addressed in some studies, but the number of subjects converting to diabetes has been limited and the follow-up time relatively short. The MPP offers sufficient power to test this hypothesis as 30,000 individuals have been followed for a median of 22 years. Thus, we tested the ability of common variants in *TCF7L2* (rs12255372; rs7903146), *PPARG* (P12A), *ENPP1* (K121Q), *CAPN10* (SNP43), *IRS1* (972C>T), *UCP2* (−866G>A), *PTPN1* (rs1044498), *PGC1* (G482S) to predict overt T2D in 7,061 individuals with DNA and follow-up data available thus far. Altogether 22,000 of the original participants will be restudied.
Materials and Methods: 4392 men and 2669 women (age 46±6 yrs, BMI 24±3.4 kg/m^2; 24.5% with IFG/IGT) were included. Genotyping was performed using the IPLEX system adopted to mass spectroscopy on a Sequenom platform. It was not possible to multiplex all potential candidate genes in the same setting, so other candidate genes will be added in the future, e.g. *KCNJ11*.
Results: In total, 1422 (20.1%) persons converted to T2D; of them 1072 (24.4%) men with a mean 25-year and 350 (13.1%) women with a mean 18-year follow-up period. In Cox regression analyses adjusted for age, sex and BMI, the *PPARG* PP-genotype (Hazard ratio 1.20, 95%CI 1.05–1.38, P=0.0045) and the T-allele of both *TCF7L2* (1.30, 1.17–1.45, $P=1.8 \times 10^{-6}$ and 1.42, 1.28–1.58, $P<10^{-10}$, for rs12255372 and rs7903146, respectively) increased the risk of future T2D. Furthermore, in the multivariate analyses *TCF7L2* (rs7903146) and *PPARG* (P12A) (1.42, 1.27–1.57, $P<10\times^{-10}$ and 1.23, 1.09–1.40, P=0.00058) were independent predictors of T2D. None of the other common variants in *ENPP1, CAPN10, IRS1, UCP2, PTPN1* and *PGC1* genes were associated with increased risk of T2D.
Conclusion: These data from a large prospective study with 22 years follow up clearly demonstrate that common variants in the *TCF7L2* and *PPARG* genes predict future T2D and can be considered true T2D susceptibility genes.

0022

The transcription factor 7-like 2 gene (*TCF7L2*) is strongly associated with type 2 diabetes in the Botnia Study

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Background and Aims: Genetic complexity of type 2 diabetes (T2D) is demonstrated by the fact that so far no susceptibility gene has confidently been associated with the disease in all studied populations. Recently, single nucleotide polymorphisms (SNPs) in *TCF7L2* were found to be strongly associated with T2D in Icelandic and two other populations. Our aim was to investigate the putative role of *TCF7L2* in the Botnia study.
Materials and Methods: Two SNPs in *TCF7L2* (rs7903146, rs12255372) were genotyped in 716 controls (m/f 346/370, age 54±11 y, BMI 25.9±3.7 kg/m^2), 751 T2D patients (m/f 399/352, age 60±10 y, BMI 28.9±4.8 kg/m^2) and 2293 non-diabetic individuals participating a prospective follow-up (1051 men/1242 women, age 45±14 yrs, BMI 25±4 kg/m^2). All individuals originate from the Botnia region in Western Finland and genotyping was performed by allelic discrimination on ABI 7900. During a mean follow-up period of 6 years 132 individuals developed T2D.
Results: The earlier described "at risk" T-alleles (minor alleles) of both SNPs were more frequent in T2D patients compared to controls (rs7903146: 41.0 vs. 31.0%, p=0.000097; rs12255372: 39.0 vs. 29.9%, p=0.00027). The T-allele carriers had increased risk of T2D as compared to homozygous major allele carriers (rs7903146: OR 1.54 [1.24–1.92], p=0.00010, rs12255372: OR 1.50 [1.20–1.86], p=0.00028). Carriers of the at-risk alleles of both SNPs had an even higher risk of T2D (OR 2.38 [1.86–3.05], p<0.000001). In the non-diabetic subjects participating in the Botnia prospective study, the T-allele carriers of rs7903146 had higher fasting- (5.60±0.58 vs. 5.54±0.58 mmol/l, p=0.031) and 2 h-plasma-glucose (6.45±1.6 vs. 6.20±1.6 mmol/l, p=0.00028) as well as lower insulinogenic index (4.91±3.9 vs. 5.36±4.4, p=0.022) and disposition index (4.35±3.3 vs. 4.75±3.8, p=0.016) at baseline. Moreover, the homozygous rs7903146 TT-genotype carriers were leaner compared to the other genotype carriers (24.2 vs. 25.7 kg/m^2, p=0.0052). Finally, already at baseline the individuals with IGT or IFG had a higher frequency of the risk genotypes (rs7903146: 39.6 vs. 34.1%, P=0.012; rs12255372: 36.1 vs. 31.1%, P=0.018) and the T-allele of rs7903146 was associated with increased risk of developing T2D among individuals with IFG at baseline (2.64 [1.09–6.42], p=0.032).
Conclusion: Variants in *TCF7L2* are strongly associated with T2D and predict morbidity to T2D in the Botnia study. Our results point at a role for *TCF7L2* in regulation of insulin secretion and glucose tolerance.

0023

Refinement of the association between the *TCF7L2* gene and type 2 diabetes in a West African population

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We recently described association between the Transcription Factor 7-like 2 gene (*TCF7L2*; formerly *TCF4*) and type 2 diabetes (T2D) with a population attributable risk (PAR) of 21%. We reported the association of three markers, the composite allele X of microsatellite DG10S478 and the T alleles of SNPs rs12255372 and rs7903146, based on an initial discovery in Iceland and subsequent replication in Danish and European American cohorts. The strength of association of these highly correlated markers in Caucasian populations was comparable so we recommended that all three should be genotyped in any replication effort.
In an attempt to replicate and refine this association, we genotyped these markers in a more genetically diverse West African cohort, consisting of 618 T2D patients and 434 controls derived from the Africa America Diabetes Mellitus Study. Following adjustment for relatedness and ethnic origin, we replicated the association of allele T of rs7903146 with T2D (Relative Risk=1.45 [1.20,1.76], $P=7.3\times10^{-5}$, PAR=22.2%); however, the other two markers were not significantly associated.
The West African cohort has therefore enabled us to define allele T of rs7903146 as either the risk variant itself or the closest correlate of a still unidentified risk variant. This

refinement was possible because the T allele of rs7903146 occurs almost exclusively on chromosomes carrying both allele X of DG10S478 and allele T of rs12255372 in populations with European ancestry, while this is not the case in the West African cohort, where it occurs with both alleles of DG10S478 and rs12255372. This is consistent with the observation that T is the ancestral allele of rs7903146, whereas allele X of DG10S478 and allele T of rs12255372 are both derived relative to the chimpanzee reference sequence. More generally, this finding is also consistent with the expectation that non-African populations tend to carry only a subset of the variants present in African populations.

0024

A KLF9 promoter variant that is predicted to affect TCF7L2 binding is associated with type 2 diabetes

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Background and Aims: We investigated contribution of Kruppel-Like Zinc Finger (KLF) transcription factor family genes to type 2 diabetes (T2D), since they regulate genes involved in cell proliferation and differentiation in several different tissues (including pancreatic beta-cells). In particular, KLF2, 5, 6, 7 and 15 were shown to regulate expression of genes involved adipose differentiation.
Materials and Methods: We genotyped 51 SNPs of 12 KLFs (minor allele frequency >5%) in a first case-control set of 365 T2D French Caucasian subjects with at least one affected first-degree relative (196/169 M/F, age at diagnosis 45±11 years) and 363 normoglycaemic subjects (136/227 M/F, age at examination 59±13 years) using Taqman (ABI), SNPlex (ABI) or Lightyper technology (Roche). SNPs showing a trend for association were analyzed in a second set consisting of 750 T2D subjects (455/295 M/F, age at diagnosis 48±10 years) and 741 normoglycaemic subjects (294/447 M/F, age at examination 53±6 years).
Results: In the first set, 13 SNPs showed a trend for association with T2D and these were further analyzed in the second set. A KLF7 intron 3 variant associated with T2D ($P<0.01$). Moreover, a KLF9 promoter variant showed a trend towards T2D association in both sets ($P=0.03$), which needs to be replicated in additional samples. In-silico analysis (Genomatix) of this promoter variant predicted that either TCF1 or TCF7L2 transcription factor binding is lost when the variant allele is present. We are now analysing TCF1/TCF7L2 binding to this variant by gelshift experiments and chromatin-immunoprecipitation.
Conclusion: Besides the previously reported T2D association of KLF11, our study suggests that KLF7 (confirming earlier findings in the Japanese population) and KLF9 may be associated to T2D. No contribution of other KLF-transcription factor variants was observed in the French population. The potential association of the KLF9 promoter variant with T2D suggests that KLF9 is a target of TCF7L2 and may be involved in the signaling pathway of this strong T2D susceptibility gene.
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OP 5

Beta cell differentiation and neogenesis

0025

Function of the transcription factor RFX3, a novel link between cilia and development of the endocrine pancreas

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Background and Aims: In mammals there are five members of the RFX family of transcription factors. While RFX5 plays a well-defined role in the immune system, the functions of RFX1–RFX4 remain largely unknown. We recently found that RFX3 plays a key role in controlling the expression of genes required for the formation and function of cilia. As consequence of defects in nodal cilia, RFX3 deficient mice exhibit frequent left–right asymmetry defects leading to a high rate of embryonic lethality and situs inversus in surviving adults.
Results: We now show that RFX3 is also expressed specifically in the islets of Langerhans and that, in the absence of RFX3, islet morphogenesis is severely perturbed. The rare RFX3-deficient mice that survive to adulthood exhibit a reduced tolerance to glucose injection, a marked reduction in the size of pancreatic islets, reduced insulin production and defective glucose-stimulated insulin secretion. These abnormalities result from a developmental

defect leading to an alteration in the cellular composition of the islets during embryogenesis. By embryonic day 19, the islets in RFX3-deficient mice contain considerably less insulin-producing β cells, glucagon-producing α cells and ghrelin-producing cells, whereas there is a marked increase in the number of pancreatic peptide (PP)-producing cells.
Conclusion: This skewed cell composition of the islets suggests that RFX3 is implicated in one or more cell fate decisions in the developing endocrine pancreas. Finally, we have examined expression in the pancreas of known or suspected RFX3 target genes coding for cilia related proteins. We find that expression of two of these genes, *D2lic* and *Tg737* is significantly reduced in the pancreas, thereby providing a link between cilia and the regulation of pancreatic islet cell development.
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0026

Regulation of Pax6 and Pax6(5a) in pancreatic beta cells

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Background and Aims: The transcription factor Pax6 is an important regulator of alpha- and beta cell function. These cell types produce and release the hormones glucagon and insulin, which are reciprocally regulated in response to glucose. In alpha cells Pax6 is important for activation as well as for insulin dependent inhibition of the glucagon synthesis. The influence of this transcription factor in beta cells on insulin gene expression is discussed controversially. Also the importance of alternative splicing and the function of the resulting Pax6(5a) in islet cells is not understood. We investigated the regulation of both isoforms in pancreatic beta cells to analyse whether these factors are involved in the regulation of insulin biosynthesis.
Materials and Methods: Gel shifts and chromatin immunoprecipitations were done to identify DNA binding sites of Pax6 within the rat insulin gene II promoter. After treatment of Insulinoma cells with different concentrations of glucose or H_2O_2, alteration of the intracellular localisation was visualised by fluorescence microscopy. The mRNA amount of Pax6(5a) was detected by RT–PCR. The effect of the exon 5a on interaction with BETA2 and on Pax6 regulated insulin gene expression was analysed using GST capture assay and the Dual Luciferase Assay system respectively. Furthermore in vitro kinase assays were performed.
Results: Pax6 is able to bind to insulin promoter cis-elements not similar to PISCES. We observed an augment nuclear localisation of EGFP-Pax6 and EGFP-Pax6(5a) in INS-1 cells under low glucose concentrations and during oxidative stress. The isoform Pax6(5a) is not able to interact with BETA2 and to suppress insulin gene expression to the same extent as Pax6. Furthermore we demonstrate that Pax6 and Pax6(5a) are phosphorylated by CaMK and CK2.
Conclusion: At low concentrations of glucose and enhanced amounts of H_2O_2 Pax6 acts as a negative regulator of insulin gene expression. Further Pax6 activity is modulated via alternative splicing and phosphorylation by CaMK and CK2.

0027

P27 regulates the transition of beta cells from quiescence to proliferation

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Background and Aims: Diabetes results from an inadequate mass of functional beta cells. Such inadequacy could result from loss of beta cells due to an immune assault or the inability to compensate for insulin resistance. Thus, mechanisms that regulate the number of beta cells will be key to understanding both the pathogenesis of diabetes and for developing therapies. In this study we show that cell cycle regulator, p27 plays a crucial role in establishing the number of beta cells formed before birth.
Methods: Expression patterns of islet transcription factors and islet hormones were analyzed by fluorescent immunohistochemistry on wildtype and p27−/− littermate mice, aged E10.5 to adulthood. Beta cell mass was measured by multiplying the insulin/area ratio (quantified by insulin area divided by total tissue area) by the mass of the pancreas. Plasma levels were measured, and interperitoneal glucose tolerance and insulin tolerance tests were performed on p27−/− and WT mice aged 10–12 weeks, after 6–16 hour fasts. The statistical significance of differences was measured by unpaired student t-test.
Results: We show that p27 accumulates in terminally differentiated beta cells during embryogenesis. Disabling p27 allows terminally differentiated beta cells that are normally quiescent during embryogenesis to reenter the cell cycle and proliferate. As a consequence, excess beta cells are generated in the p27−/− mice, doubling their beta cell mass at birth. The early postnatal expansion of beta cell mass was unaffected in p27−/− mice, indicating that the main function of p27 is to maintain the quiescent state of newly differentiated beta cells generated during embryogenesis. The expanded beta cell mass

was accompanied by increased insulin secretion, however, the p27−/− mice were glucose intolerant as these mice were insulin insensitive. To assess the role of p27 to affect regeneration of beta cells in models of diabetes, p27−/− mice were injected with streptozocin. In contrast to control mice that displayed elevated blood glucose levels, p27−/− mice showed decreased susceptibility to develop streptozotocin-induced diabetes. Furthermore, beta cells retained the ability to reenter the cell cycle at a far greater frequency in p27−/− mice after develop streptozotocin-induced diabetes compared to wild-type littermates.

Conclusion :This data indicates that p27 is a key regulator in establishing beta cell mass and an important target for facilitating beta cell regeneration in therapies for diabetes.

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0028

Extracellular matrix increases rat but not human beta cell proliferation

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Background and Aims: Inducing proliferation of beta cells in vitro is attractive for both transplantation and biological studies. The aim of the study was to investigate a possible role of extracellular matrix (ECM) in inducing cell proliferation of rat and human beta cells.

Materials and Methods: Autofluorescence FACS-sorted rat pancreatic beta cells were plated on poly-L-lysine (PLL) or on matrix produced by 804G rat bladder carcinoma cell line (804G-ECM) in continuous presence of BrdU. FACS purification of human beta cells involved exclusion of ductal cells labelled with anti CA19-9 and dead cells stained with 7-aminoactinomycin D, with beta cells sorted by virtue of their high zinc content using Newport Green, yielding a population with 90% beta cells. Human beta cells were plated either on lawns of NH_4OH-lysed HTB9 human bladder carcinoma cells or on 804G-ECM or plastic in presence of BrdU during the last five days. Cell spreading was monitored microscopically, and insulin secretion measured after static incubation (1 h at 2.2 mM glucose +1 h at 22.2 mM glucose) by radioimmunoassay. Cell replication was determined using BrdU incorporation and beta cells identified by insulin immunofluoresence. Data are mean±SEM of at least 3 independent experiments.

Results: As previously shown, rat beta cells spread on 804G-ECM but not on PLL. Rat beta cells proliferated on PLL and 804G-ECM with a significant beneficial effect of the matrix at all time points. After 2 days, percentage of BrdU positive beta cells was 1.8±0.4 vs. 4.6±0.5 (P=0.006) on PLL vs. 804G-ECM respectively. After 5 days of continuous BrdU labelling, these values were 25.18%± 1.85 vs. 41.5%±3.6 (P=0.007). The results for the purified human beta cells were strikingly different. After one or two weeks of culture most of the human beta cells were spread on lysed HTB9 lawns but not on PLL or on 804G-ECM. However, little if any beta-cell replication was observed under any condition (n=6). The method used for sorting human beta cells is not in cause because sorted human beta cells responded well to glucose (stimulation index high vs. low glucose=6.1±0.9) and no apoptosis was observed in these preparations. Furthermore rat beta cells purified with the same Newport Green sorting method used for human cells, can proliferate and show similar stimulation by 804G-ECM. Intact human islets were also plated on lysed HTB9 lawns or on plastic. There were BrdU positive cells in islets; however, after confocal analysis, we observed that BrdU positive cells were almost always insulin negative with <1 BrdU insulin double positive cell/islet.

Conclusion: It is concluded that rat beta cells can proliferate in vitro and this is increased significantly by ECM. By contrast, sorted human beta cells do not replicate to any significant extent in vitro regardless of the substratum. Our data do not exclude, however, that division of human beta cells is accompanied by rapid loss of insulin.

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0029

Expansion of mesenchymal cells from human pancreatic islets of Langerhans and their "*in vitro*" differentiation in insulin secreting cells

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Background and Aims: "*In vitro*" development of cells able to produce and secrete insulin in a physiological manner is crucial to produce a proper cell-therapy as a cure for type I diabetes. Aims of our work have been the development of cell culture strategies for expansion of proliferating cells with potential beta-cell differentiation

capacity obtained from human pancreatic islets and the characterization of such processes.

Material and Methods: Freshly, highly purified human pancreatic islets from 6 normal cadaveric multi-organ donors were cultured in 100 mm tissue-dishes in presence of RPMI 1640 medium containing 10% FCS (growth medium-GM) at 37°C in 5% CO_2 to obtain a proliferating cell population which was expanded. To induce beta-cell differentiation, the proliferating cells were plated in serum-free RPMI supplemented with BSA, transferrin, insulin and sodium selenite as a differentiation medium (DM). Morphological and molecular characterization of proliferating and differentiating cells was performed by electron-microscopy, FACS, immunofluorescence and Real-Time-PCR

Results: The human islet preparations, with no sign of dissociation, were seeded in GM and, after 48 hours, a proliferating cell population migrated out of adherent islets. These cells were harvested with trypsin every 8–10 days and after 7 months, approximately 10^{10} cells were obtained from each original islet seeded, before showing evident signs of senescence. The expression of CD90, CD73, CD13, CD44, CD49e, CD49b, Nestin, αSmooth-Muscle-Actin, Slug e Snail1 analyzed by FACS, immunofluorescence and Real-Time-PCR indicated a mesenchymal phenotype of our proliferating cells. Such mesenchymal-like cells, when cultured in DM, generated "islets-like" aggregates within 10–20 days. Electron microscopy analysis of such aggregates showed mesenchymal-like cells in the outer layers surrounding a "core" of cells with a well developed Golgi apparatus characterized by enlarged cisternes, and beta-cell like secretory granules. Immunofluorescence analysis showed the presence of C-peptide in these cellular aggregates and, accordingly, in vitro insulin secretion in the presence of 3.3 mM glucose was detected by RIA. Real-Time-PCR analysis, showed a significant induction of gene expression of insulin, glucagon, somatostatin, Ngn3, NeuroD1, Isl1, Nkx2–2, Nkx6–1, IRS1 and synaptophysin, confirming an acquired endocrine pancreatic phenotype of our aggregate.

Conclusion: We obtained from human pancreatic islets a proliferating mesenchymal-like cell population which was expanded and induced to re-differentiate into insulin-secreting cells.

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Pancreatic islet-cell differentiation from adult hepatic stem cells directed in vivo by Ad/AAV hybrid vector-mediated expression of NGN3, Beta2, and RIPE3b1

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Background and Aims: Recent work showed that adenovirus—mediated ectopic expression of PDX-1 induced differentiation of insulin producing cells in mouse liver. PDX-1 also activated exocrine genes in these cells, resulting in destruction of insulin producing cells. Using BETA2 (NeuroD), downstream factor of PDX-1, specific differentiation of endocrine islet—cells was demonstrated. We hypothesized that simultaneous expression of multiple transcription factors involved in islet development may enhance the differentiation of stem cells.

Materials and Methods: Expression cassettes carrying rat PDX-1 (852 bps), mouse BETA2 (1,074 bps), NGN3 (645 bps), and/or RIP3b1 (1084 bp) with IRES were inserted into Adenovirus/adeno-associated virus (Ad/AAV) hybrid vectors, in which the AAV integration machinery was incorporated into adenoviral vectors. Male CD1 mice were injected with 2×10^9 PFUs of Ad/AAV hybrid vectors through the tail vein after induction of diabetes by intraperitoneal administration of streptozotocin. Daily blood glucose levels were monitored and intraperitoneal glucose tolerance test were performed in mice showing normalization of blood glucose level.

Results: Hyperglycemia in diabetic mice was ameliorated after injection of Ad/AAV hybrid vectors carrying bicistronic constructs. Intraperitoneal glucose tolerance test showed near normal responses. We detected the activation of insulin and other islet-specific transcripts, including glucagon, somatostatin, islet-amyloid peptide, and PDX-1. Immunocytochemistry confirmed the presences insulin and glucagon producing cells at the hepatic periportal area.

Conclusion: Our results suggest that directed differentiation of adult stem cells by the delivery of cell-lineage specific transcription factors may be a useful strategy for the treatment of hormonal insufficiency.

OP 6

Insulin and nutrient signaling pathways

0031

Identification of *TXNIP* as a marker and regulator of glucose homeostasis in humans

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Background and Aims: To identify novel pathways regulated by insulin in skeletal muscle using genome-wide expression profiling in human skeletal muscle.
Materials and Methods: We performed gene expression profiling in muscle biopsies taken before and after a euglycemic, hyperinsulinemic clamp using oligonucleotide (Affymetrix HGU133A GeneChip®) microarrays in 6 non-diabetic individuals. For replication we performed real time-PCR of genes significantly influenced by insulin in 96 young non-diabetic humans. Functional studies included studies of effect of insulin and glucose in human adipocytes, overexpression (lentiviral transduction) and siRNA gene silencing in 3T3-L1 cells and human skeletal muscle cells.
Results: Three genes were consistently influenced by insulin in all protocols *G0S2*, was strongly induced by insulin (1.65±0.17 vs. 6.01±0.62, $P<1\times10^{-16}$), while *TXNIP* (thioredoxin interacting protein) (0.18±0.02 vs. 0.06±0.01, $P<1\times10^{-14}$) and *BCL6* (0.27±0.02 vs. 0.22±0.03, $P<1\times10^{-5}$) were strongly repressed by insulin. *TXNIP* expression was also consistently elevated in individuals with T2DM as compare to NGT (1558.5± 141.24 vs. 973.71±122.42, P=0.01 & 14211.93±1241.44 vs. 10181.72±673.09, P=0.02) in two previously published microarray datasets of human muscle. Moreover, *TXNIP* expression is inversely correlated with insulin-stimulated glucose metabolism in NGT or IGT subjects (N=25, $r=-0.67$, $P<0.001$), but lost in the patients with T2DM (N=18, $r=-0.21$, P=0.41). While insulin suppressed, glucose upregulated *TXNIP* expression by >1.5-fold ($P<0.05$) at 25 mM glucose concentration in a human adipocytes cell line. A two-fold forced overexpression of *TXNIP using* lentiviral transduction results in both diminished basal (−59%, $P<0.02$) and insulin stimulated (−28%, $P<0.005$) glucose uptake while forced reduction of *TXNIP* expression by siRNA gene-silencing achieves the opposite effect and enhances both basal (+157%, $P<0.02$ &+48%, $P<0.01$) and insulin stimulated (+61%, $P<0.001$ &+49%, $P<0.01$) glucose uptake in 3T3-L1 cell line and human skeletal muscle cells respectively.
Conclusion: TXNIP represents a novel pathway regulated by glucose and insulin and could play a central role in the pathogenesis of metabolic defects leading to human T2DM.

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siRNA based gene silencing reveals specialized roles of IRS-1 and IRS-2 in glucose and lipid metabolism in human skeletal muscle

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Background and Aims: Insulin receptor substrates (IRS) 1 and 2 control metabolic and mitogenic responses and participate in insulin action in human skeletal muscle. Defects in IRS-1 or IRS-2 tyrosine phosphorylation contribute to insulin resistance, impaired glucose tolerance and Type 2 diabetes. We determined the importance and relative contribution of IRS-1 and IRS-2 to diverse, insulin-mediated metabolic responses in human skeletal muscle.
Materials and Methods: siRNA-mediated specific gene silencing was used to suppress IRS-1 and IRS-2 in primary human skeletal muscles cells to determine whether there are specialized or redundant roles of these insulin signal transducers on insulin-mediated glucose and lipid metabolism. *si*RNA was transfected into 80% confluent cells and differentiation was induced the following day.
Results: In myoblasts, depletion of IRS-1 impaired cell growth and differentiation. Therefore, the effect of IRS ablation was determined in myotubes. IRS-1 silencing completely abolished insulin-mediated phosphorylation of Akt at Ser^{473}, AS160, and FoxO1/FoxO4 and partly (50%) decreased Akt Thr^{308} phosphorylation. Conversely, IRS-2 suppression was without effect on phosphorylation of Akt at Ser^{473}, AS160 and, FoxO1/FoxO4, but dramatically decreased Akt Thr^{308} phosphorylation. The effect of *si*RNA

silencing of IRS-1 or IRS-2, was determined on glucose uptake and glycogen synthesis in differentiated myotubes. In control cells (untransfected cells or cultured cells transfected with a scrambled *si*RNA construct), insulin increased glucose uptake 2-fold (P<0.01). Insulin action on glucose uptake was prevented in IRS-1 depleted myotubes and preserved in IRS-2 depleted myotubes. Insulin increased glucose incorporation into glycogen 2.2-fold in control cells (P<0.01). IRS-1 silencing inhibited insulin action on glycogen synthesis (p<0.001), whereas IRS-2 silencing had no effect. In control cells, insulin increased palmitate uptake (2-fold; P<0.01) and decreased beta-oxidation (2.8-fold; P<0.01). IRS-2 suppression increased basal palmitate uptake, whereas IRS-1 depletion was without effect. Insulin action on palmitate uptake was unaltered by IRS-1 silencing and reduced by IRS-2 silencing. Basal beta-oxidation was decreased 220% (P<0.02) in IRS-1 depleted cells. Insulin action on beta-oxidation was preserved in IRS-1 depleted cells, whereas IRS-2 depletion completely abolished insulin action.

Conclusion: We provide evidence for specificity in insulin signaling responses governing myotube formation and glucose/lipid metabolism. Insulin signaling pathways involving IRS-1 are necessary for myotube formation, glucose uptake, and phosphorylation of Akt and AS160 whereas IRS-2 is dispensable. In contrast, insulin signaling pathways involving IRS-2 are necessary for lipid uptake and metabolism.

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siRNA based gene silencing reveals specialised roles for Akt1 and Akt2 on glucose and lipid metabolism in human skeletal muscle

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Background and Aims: Insulin-mediated activation of PI3-kinase and Akt has been implicated in glucose and lipid metabolism. The complexity of the pathway is magnified by existence of multiple isoforms of these signaling transducers. Genetic studies in knockout mice have shown Akt1 is involved in growth and Akt2 is involved in maintaining normal glucose homeostasis. The objective of this study was to determine the importance and relative contribution of Akt1 and Akt2 to diverse, insulin-mediated metabolic responses in human skeletal muscle.

Materials and Methods: We have used *si*RNA-mediated specific gene silencing to suppress Akt1 and Akt2 in primary human skeletal muscle cells to determine whether there are specialized or redundant roles of these targets in insulin-mediated signal transduction, as well as in glucose and lipid metabolism. Transfection was performed on day 3 of differentiation.

Results: Depletion of Akt2 in myoblasts impaired cell growth and differentiation, whereas Akt1 was without effect. Therefore, the effect of Akt isoform silencing was studied in myotubes. Akt2 silencing abolished insulin-mediated phosphorylation of Akt at Ser^{473}, AS160, and FoxO1/FoxO4 and partly decreased (33%; P<0.001) Akt Thr^{308} phosphorylation. Conversely, Akt1 suppression was without effect on phosphorylation of Akt at Ser^{473}, AS160 and, FoxO1/FoxO4, but decreased (55%; P<0.01) Akt Thr^{308} phosphorylation. The effect of *si*RNA silencing of Akt1 and Akt2, was determined on glucose uptake and glycogen synthesis 4 days after transfection. In control cells insulin increased glucose uptake 1.8-fold (P<0.01). This insulin-mediated increase was prevented in Akt2 depleted cells (P<0.05), but preserved in cells depleted of Akt1. Insulin increased glucose incorporation into glycogen 3.2-fold in control cells (P<0.001). Akt 2 ablation inhibited insulin action on glycogen synthesis (P<0.01), whereas Akt1 silencing was without affect. In control cells, insulin increased palmitate uptake 2.6-fold (P<0.01) and decreased beta-oxidation 3.5-fold (P<0.001). Akt1 suppression increased basal palmitate uptake 1.6-fold (P<0.05), whereas Akt2 depletion was without effect. Insulin action on palmitate uptake was increased 2-fold by Akt2 silencing (P<0.05) and unaltered by Akt1 silencing. Basal beta-oxidation was increased 1.8-fold (P<0.001) in Akt1 depleted cells and decreased 0.3-fold (P<0.05) in Akt2 depleted cells. Insulin action on beta-oxidation was preserved in Akt2 depleted cells.

Conclusion: Using *si*RNA-mediated gene silencing, we provide that insulin signaling pathways involving Akt2 are required for myotube formation, glucose uptake, and phosphorylation of Akt and AS160. In contrast, insulin signaling pathways involving Akt1 are necessary for lipid uptake and metabolism. Akt2 can partly compensate for Akt1 to mediate lipid metabolism.

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Reduced phosphorylation of proline-rich PKB/Akt substrate PRAS40 under conditions of high-fat diet induced insulin resistance

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Background and Aims: The number of people diagnosed with type 2 diabetes mellitus (T2DM) is rising rapidly. Insulin has its action via the insulin receptor, which results in the activation of downstream factors phosphatidylinositol 3′-kinase (PI3K) and its substrate protein kinase B (PKB/Akt). An early clinical hallmark of T2DM is insulin resistance. This condition is associated with decreased activation of PI3K and PKB/Akt. Activation of PKB/Akt has been implicated in different cellular processes eg. glucose uptake, cell survival and stress signaling, however the downstream protein substrates regulating these responses are poorly characterized. In the current study, novel endogenous substrates for PKB/Akt were characterized in different insulin target tissues under normal conditions and under conditions of insulin resistance.
Materials and Methods: To this end, adult male Wistar rats were put on a high-fat diet (HFD) or low-fat diet (LFD) for 7 weeks. 30 min prior to sacrifice, animals received insulin or saline via i.p. injection.
Results: In all examined tissues (cardiac tissue, hepatic tissue, skeletal muscle) of LFD-fed rats, insulin strongly induced the phosphorylation of a ~30-kDa (p30) and a ~40-kDa (p40) protein. Only in skeletal muscle and cardiac muscle, phosphorylation of a ~160-kDa (p160) was observed. p30 was identified as ribosomal protein S6 and p160 as AS160, which are involved in the regulation of protein synthesis and GLUT4 mediated glucose uptake respectively. p40 was identified as proline-rich PKB/Akt substrate 40 kDa (PRAS40). PRAS40 has been implicated in protection of neural cells from apoptosis, however its role in insulin action remains elusive.
In vivo phosphorylation of PRAS40 on Thr246 after insulin stimulation was observed in adipose tissue, cardiac muscle, skeletal muscle and hepatic tissue. *In vitro* experiments confirmed PI3K dependent signaling with the use of PI3K inhibitor wortmannin. Immunohistochemical staining showed nuclear localization of phosphorylated PRAS40 under physiological conditions in LFD-fed animals. *In vitro* immunofluorescent staining also displayed nuclear localization of phosphorylated PRAS40. Insulin stimulation of cultured cells increased nuclear localization of phosphorylated PRAS40, next to detection of a small amount of phosphorylated PRAS40 in the cytoplasm. Finally in HFD-induced insulin resistant animals, phosphorylation of PRAS40 was significantly reduced compared to LFD-fed animals in all examined tissues. The reduction of phosphorylated PRAS40 in HFD-fed animals was also accompanied with reduced phosphorylation of PKB/Akt.
Conclusion: PRAS40 has been identified as a novel target of in vivo insulin action. Phosphorylation of PRAS40 is increased by insulin in different insulin target tissues (skeletal muscle, cardiac muscle, liver, adipose tissue). HFD-induced insulin resistance induced results in reduced PRAS40 phosphorylation. Future experiments are planned to identify the role of PRAS40 in insulin action.
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0035

Insulin differentially affects glucose metabolism in human endothelial- and smooth muscle cells

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Background and Aims: Diabetes mellitus and insulin resistance are characterized by vascular endothelial cell dysfunction. The molecular mechanisms linking endothelial glucose metabolism with diabetes-associated vascular disease and insulin resistance have, however, not been fully elucidated. So far, available data are controversial and are primarily restricted to animal endothelial cells. Therefore, this study aimed at analysis of the effects of insulin on glucose metabolism of human micro- and macrovascular endothelial cells and human smooth muscle cells.
Materials and Methods: Human umbilical vein- (HUVECs), adult vein- (HAVECs), aortal- (HAECs), and retinal (HRECs) endothelial cells as well as smooth muscle cells (HSMCs) were incubated with insulin (0.3 nmol/l and 1 μmol/l) or LiCl (5 and 10 mmol/l) and evaluated for glucose transport (^{3}H-2-deoxy-glucose accumulation), glycogen synthesis (net glucose incorporation into glycogen), glycogen content (quantification of glucose from glycogen), proliferation (^{3}H-thymidine incorporation), and protein expression (Western blots). Most experiments were performed in medium containing 5.5 mmol/l glucose, some in the presence of 30 mmol/l glucose.

Results: In HUVECs (n=6), insulin increased ($p<0.05$) proliferation (+63±13%), endothelial NO synthase expression (+61±23%) and Akt (protein kinase B) phosphorylation (+101±38%), the latter, however, to a lesser extent than in HSMCs (+1093±500%). Insulin stimulated glycogen synthesis in HSMCs from 1866±358 to 3248±702 fmol/cell/hour ($p<0.05$), but had no effect on glucose transport, glycogen synthesis, and glycogen content in the different endothelial cell species. Nevertheless, the rates of glycogen synthesis in endothelial cells were increased ($p<0.05$) by ***i)*** LiCl, an inhibitor of glycogen synthase kinase-3β (up to +105±25%), ***ii)*** overnight glucose starvation (+556±38%), and ***iii)*** incubation (48 h) with 30 mmol/l glucose (+66±10%). But even in HAECs (n=8), preincubated with high glucose, insulin neither stimulated glucose transport (339±49 versus 337±36 cpm[^{3}H-2d-glucose]/10^6cells/10minutes, n.s.) nor glycogen synthesis (694±98 versus 707±71 fmol/cell/hour, n.s.).

Conclusion: Insulin increased endothelial proliferation and endothelial NO-synthase expression and activated Akt, a key regulator of glycogen metabolism. Incubation with LiCl (an inhibitor of glycogen synthase kinase-3β), overnight glucose starvation, and preincubation with high glucose stimulated glycogen synthesis in endothelial cells. Exposure to insulin, however, had no effect on glucose transport, glycogen synthesis, and glycogen content of human micro- and macrovascular endothelial cells. A recently described high glucose-induced *de novo* insulin sensitization of HAECs could not be confirmed in our study. In contrast to smooth muscle cells, insulin, apparently, has no major effects on human endothelial glucose metabolism, independent of the cells' vascular origin (micro- versus macrovascular).

0036

Amino acids inhibit glucose oxidation independently of the mTOR/p70S6-kinase pathway in isolated rat skeletal muscle

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Background and Aims: Mammalian target of rapamycin (mTOR) and its downstream target p70S6-kinase (p70S6K) have been proposed to be implicated in the development of insulin resistance. This relates to the finding that activation of the mTOR/p70S6K pathway by amino acids causes serine/threonine phosphorylation of insulin receptor substrate-1, which disrupts downstream signaling events and eventually impairs glucose utilisation. The present study challenges this concept by showing that excess supply of amino acids affects glucose metabolism of isolated skeletal muscle predominantly via an other mechanism.

Materials and Methods: Isolated specimens of skeletal muscle from healthy male Sprague–Dawley rats were incubated for 2 h with 2.5%, 5%, or 10% (vol/vol) of an amino acid mixture (Aminoplasmal–10%® AP). Where indicated, 20 or 40 nmol/l rapamycin, a specific inhibitor of the mTOR/p70S6K pathway, was added. During the last hour of the experiment, rates of glucose oxidation and glycogen synthesis were measured in the absence or presence of 10 nmol/l insulin. The activity of p70S6K is given as % of the total kinase being in the phosphorylated (=active) state as detected by Western Blot analysis.

Results: In line with previous reports, the activity of p70S6K in isolated skeletal muscle decreased in response to rapamycin (% of kinase in phosphorylated state: control, 95±3; 20 nmol/l rapamycin: 67±8; 40 nmol/l rapamycin: 60±8; $p<0.01$ each vs. control) and increased in response to amino acids (% of kinase in phosphorylated state: control, 53±4; 2.5%AP, 64±3, $p<0.05$; 5%AP, 69±3, $p<0.002$; 10% AP, 76±5; $p=0.056$, ns). Amino acid-induced activation of p70S6K was accompanied by a distinct dose-dependent reduction of insulin-stimulated glucose oxidation (μmol glucose converted into $CO_2*g^{-1}*h^{-1}$: control, 3.6±0.2; 2.5%AP: 2.1±0.2; 5%AP: 1.4±0.1; 10%AP: 0.8±0.1; $p<0.001$ each vs control). However, our findings argued against insulin desensitisation via the mTOR/p70S6K pathway being responsible for the observed impairment of glucose oxidation, (i) because rapamycin failed to counteract the effect of amino acids on glucose oxidation (μmol$*g^{-1}*h^{-1}$: control, 4.1±0.8; 5%AP without rapamycin: 1.5±0.2, $p<0.01$; 5%AP with 20 nmol/l rapamycin, 1.6±0.2, 5%AP with 40 nmol/l rapamycin, 1.7±0.1, ns for effect of rapamycin), (ii) because amino acids impaired also basal, i.e. insulin independent, glucose oxidation (μmol$*g^{-1}*h^{-1}$: control, 2.9±0.3; 2.5%AP: 1.4±0.2; 10% AP: 0.6±0.1; $p<0.005$ each vs control), and (iii) because insulin-stimulated glycogen synthesis, which is known to be severely blunted in insulin resistant muscle, was not affected by amino acids (μmol glucose incorporated into glycogen$*g^{-1}*h^{-1}$: control, 4.2±0.3; 2.5%AP: 4.4±0.3; 5% AP: 4.4±0.3; 10%AP: 3.7±0.3; ns vs control).

Conclusion: High concentrations of amino acids distinctly inhibit glucose oxidation in isolated native skeletal muscle, which can be attributed neither to insulin desensitisation nor to activation of the mTOR/p70S6K pathway. We speculate that the effects of excess amino acids on cellular glucose metabolism could relate to substrate competition on the level of the tricarboxylic acid cycle.

OP 7

Novel therapies – type 2 diabetes

0037

Initial safety, tolerability and glucose lowering of CS-917, a novel fructose 1,6-bisphosphatase (FBPase) inhibitor, in subjects with type 2 diabetes

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Background and Aims: CS-917 is the prodrug of a potent and specific inhibitor of the enzyme, fructose 1,6-bisphosphatase (FBPase), being developed as a potential therapy for type 2 diabetes. This rationally designed agent is activated preferentially in the hepatocyte where it acts to suppress the augmented gluconeogenesis that contributes to fasting and post-prandial hyperglycemia in these patients.
Materials and Methods: Thirty-nine subjects (30 male, 9 female) with type 2 diabetes, mean age 52–62 years, with mean fasting glucose 205–249 mg/dL, were randomized to receive one of four doses of CS-917 (50, 100, 200 or 400 mg) or placebo, orally, once daily in the morning, for 14 days, in sequential escalating dose panels. Evaluations included assessments of safety and tolerability. Measurements of pharmacokinetic and pharmacodynamic parameters were collected during 24-hour blood sampling periods and areas under the curve for relevant parameters were calculated on Days −1,+1 and+14. An additional cohort was added that received CS-917 400 mg (N=10) or placebo (N=4) QHS, evaluated for safety and tolerability.
Results: CS-917 was well tolerated in subjects with type 2 diabetes over 14 days of dosing. There were no serious adverse events. No hypoglycemia occurred. One subject (400 mg QHS) withdrew due to rash. Two subjects(400 mg QHS and 200 mg QAM) withdrew consent. No subjects reported vomiting with QAM dosing. In the 400 mg QHS group one subject reported nausea and one subject experienced vomiting on Day 1. Both episodes resolved and both subjects continued and completed the 14 day course.
CS-917 was rapidly absorbed, extensively metabolised and rapidly cleared from plasma in patients with type 2 diabetes. Maximum concentrations of the active metabolite, R-125338, were reached between 2 to 3 hours after both single and multiple doses. Mean exposure and Cmax for the parent and active metabolite were approximately linear over the range of 50–200 mg.
CS-917 treatment resulted in statistically significant lowering of post-dose fasting plasma glucose (delta $AUC_{Glucose}$(0–6 hours)), compared to placebo. Two subjects in the 400 mg morning-dose group had isolated asymptomatic elevated post-prandial lactic acid levels >4.5 mmol/L. Lactic acid profiles were not different compared to placebo for doses of 50 and 100 mg but $AUC_{lactic\ acid}$(0–6 hours) was increased versus placebo in the 400 mg group.
Conclusion: CS-917 was generally safe and well tolerated during 14 days of treatment in subjects with type 2 diabetes. The incidence of gastrointestinal side effects was low but dose related nausea and vomiting occured at 400 mg. An initial glucose lowering effect was demonstrated with no occurence of hypoglycemia. Mean lactic acid concentrations remained within normal limits but were increased versus placebo at the highest dose of 400 mg. Further exploration of the potential efficacy of CS-917 as a novel treatment for type 2 diabetes is warranted.
Supported by Sankyo Pharma Development

0038

Twelve-week efficacy and tolerability of sitagliptin, a Dipeptidyl Peptidase-4 (DPP-4) inhibitor, in Japanese patients with type 2 diabetes

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Background and Aims: Sitagliptin (MK-0431) is an orally active, potent, selective DPP-4 inhibhitor in development for the treatment of type 2 diabetes. This randomized, double-blind, placebo-controlled, parallel group study evaluated the efficacy and tolerability of sitagliptin in Japanese patients with type 2 diabetes.
Materials and Methods: Patients with type 2 diabetes either not on an antihyperglycemic agent or on an antihyperglycemic agent in monotherapy (discontinued at entry into the run-in period), entered an 8 week diet and exercise run-in period. Patients with an A1C of 6.5 to 10.0% after the run-in period were eligible to be randomized to sitagliptin 100 mg q.e. or placebo for a 12-week double-blind treatment period. The efficacy analysis was based on a modified intention-to-treat population with last observation carried forward.
Results: Patients (N=151), ages 27–69 years, were randomized 1:1 to sitagliptin (n=75) or to placebo (n=76). Mean baseline A1C was 7.5% in the sitagliptin and 7.7% in the placebo group. At week 12, A1C decreased by 0.65% in the sitagliptin group compared with an increase of 0.41% in the placebo group, for a between-treatment group difference in A1C of 1.05% (95% CI: 1.27, 0.84%; $p<0.001$). In an analysis of patients achieving A1C goal,

58.1% of patients on sitagliptin achieved an A1C of<7% compared with 14.5% on placebo. Fasting plasma glucose decreased by 22.5 mg/dL with sitagliptin and increased by 9.4 mg/dL with placebo after 12 weeks, for a between treatment difference of 31.9 mg/dL (95% CI: 39.7, 24.1 mg/dL; p<0.001). β cell function as assessed by HOMA- β index was significantly improved with sitagliptin group compared to placebo group. In a subset patients that underwent a meal tolerance test at baseline and at Week 12, 2-hour postprandial glucose decreased by 69.2 mg/dL in the sitagliptin group compared with an increase of 11.7 mg/dL on placebo group. 2-hour insulin and C-peptide were also increased after treatment with sitagliptin group versus placebo group. Treatment with sitagliptin was well tolerated with no reported adverse experiences of hypoglycemia. The overall incidence of adverse experiences reported was similar between treatment groups. Although body weight was slightly reduced in the sitagliptin group after 12 weeks (−0.1 kg), the reduction in body weight was significantly (p=0.003) greater in teh placebo group (−0.7 kg).

Conclusion: In this study, sitagliptin 100 mg q.d. in monotherapy was efficacious and well tolerated in Japanese patients with type 2 diabetes, who no reports of hypoglycemia.

0039

Addition of Sitagliptin to pioglitazone improved glycaemic control with neutral weight effect over 24 weeks in inadequately controlled type 2 diabetes

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Background and Aims: Thiazolidinediones are increasingly used in type 2 diabetes (T2DM) but may not provide sufficient glycemic control in monotherapy. This 24-wk, randomized, double-blind, placebo (PBO)-controlled study assessed the efficacy and safety of adding once-daily sitagliptin (SIT), a DPP-4 inhibitor, to patients with T2DM treated with pioglitazone (PIO) monotherapy.

Materials and Methods: After a diet/exercise run-in and an 8–14 wk dose-stable period on PIO (duration according to previous therapy) and a 2-wk single-blind PBO run-in period, patients with HbA_{1c} ≥7%–≤10% were randomized (1:1) to addition of PBO or SIT 100 mg q.d. to ongoing PIO. Mean baseline characteristics of randomized patients (N=353) including age 56 yrs (range 24–87 yrs); diabetes duration 6.1 yrs; HbA_{1c} 8.0%; fasting plasma glucose (FPG) 9.2 mmol/L; and BMI 31.5 kg/m^2 were similar between groups. Efficacy analysis was based on an all patients treated using ANCOVA.

Results: After 24-wks, addition of SIT to PIO produced significant PBO-subtracted reductions in HbA_{1c} (−0.70%, p<0.001) and FPG (−0.98 mmol/L, p<0.001). SIT effects on glycemic control were maintained over the 24-wk study. Endpoint HbA_{1c} was 7.2% and 7.8% for SIT and PBO and % of patients reaching target HbA_{1c}<7% was 45% and 23% (p<0.001), respectively. Proinsulin levels and proinsulin/insulin ratio were significantly reduced with SIT compared with PBO, suggesting improvements in β-cell function. Sitagliptin was well tolerated with no increased risk of hypoglycemia compared with PBO; a slightly higher incidence of abdominal pain was observed with SIT, with no significant differences in other GI AEs compared to PBO. Mean body weight change was not different between SIT and PBO when added to PIO.

Conclusion: In patients with T2DM with inadequate glycemic control on PIO, the addition of sitagliptin 100 mg once daily led to significant lowering of HbA_{1c} and FPG while improving some measures of β-cell function without additional weight gain and with good tolerance over a 24-wk period.

Supported by Merck & Co. Inc.

0040

The effect of the dipeptidyl peptidase IV inhibitor LAF237 on gastrointestinal function and glucose metabolism in type 2 diabetes

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Background and Aims: Incretin-based therapy is a useful adjunct for the treatment of type 2 diabetes. Administration of glucagon-like peptide-1 (GLP1) or GLP1 receptor (GLP1R) agonists has been shown to delay gastric emptying. Chronic administration of GLP1R agonists leads to weight loss. However, the effect of compounds that raise endogenous GLP1 concentrations by inhibition of dipeptidyl peptidase IV (DPP-IV) on gastrointestinal function is unknown. In addition, the relative contribution of DPP-IV induced changes in gastric function (if any), insulin secretion and/or glucagon suppression to improved glycemic control in people with type 2 diabetes is unknown.

Materials and Methods: To address these questions, we studied 14 subjects with type 2 diabetes who had no history of pre-existing gastrointestinal dysfunction. Using a double

blind, placebo-controlled crossover design, subjects were given LAF237 (50 mg bid) or placebo for 10-days in random order separated by a three-week washout. On day 9 of each treatment period, subjects ate a meal consisting of 50 g of bacon, 2 scrambled eggs labeled with ^{99m}Tc-Sulfur colloid and 75 g of Jell-O labeled with [1-^{13}C]-glucose. [6-^{3}H] glucose also was infused intravenously to enable measurement of the systemic rate of appearance of the ingested glucose. Gastric accommodation was measured on a separate study day (day 7) using single photo emission computed tomography (SPECT) and intravenous ^{99m}Tc-sodium pertechnetate.

Results: As expected, treatment with LAF237 reduced postprandial glucose concentrations (1.048±0.0105 vs. 0.941±0.0095 mol/L per 6 h, *P*=0.018) and suppressed glucagon (29695±1452 vs. 26975±1135 ng/L per 6 h, *P*=0.03). C-peptide concentrations did not differ between study days (746±92 vs. 697±60 nmol/L, *P*=0.35). LAF237 did not alter gastric emptying, gastric accommodation, or the systemic rate of appearance of the ingested glucose (table 1)

Conclusion: LAF237 does not alter gastric emptying, gastric accommodation or the rate of entry of ingested glucose into the systemic circulation in humans. Therefore, in contrast to GLP1R agonists, DPP-IV inhibitors do not lower postprandial glucose concentrations by altering the rate of nutrient absorption.

Data are shown as mean + SEM

	Meal Ra	Gastric Emptying			Gastric Accommodation		
	μmol/kg per 6 h	% ^{99m}Tc counts retained	T_{lag} (mins)	T_{50} (mins)	Fasting Volume (mL)	Initial (0 min) Fed Volume (mL)	15 minute Fed Volume (mL)
LAF237	8974±918	0.17±0.05	35±4	145±7	248.2±20.8	731.0±30.5	746.2±27.9
Placebo	8902±525	0.15±0.05	40±6	143±7	247.3±18.9	747.0±23.9	771.8±25.9
P	*0.91*	0.36	0.46	0.79	0.98	0.65	0.54

Supported by Novartis, who provided LAF237 and salary support

0041

Vildagliptin suppresses Endogenous Glucose Production (EGP) and increases beta cell function after single dose administration in type 2 diabetic (T2D) patients

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Background and Aims: Vildagliptin is a selective DPP-4 inhibitor that augments meal-stimulated levels of biologically active GLP-1 levels. Chronic vildagliptin treatment decreases postprandial glucose levels and reduces HbA_{1c} in type 2 diabetes (T2D). However, little is known about the mechanisms by which vildagliptin promotes reduction in fasting plasma glucose (FPG) concentration.

Materials and Methods: To examine the mechanisms via which vildagliptin improves glucose tolerance and reduces FPG in T2D.

Results: 16 T2D (age=48±12 y; BMI=34.4±6.7; HbA_{1c}= 9±1.2%) participated in a randomized, double-blind, placebo (PBO)-controlled, trial. On separate days patients received 100 mg vildagliptin or placebo at 5:30PM followed 30 min later by 6-h meal tolerance test (MTT) performed with double tracer technique (3-^{3}H-glucose iv and 1-^{14}C-glucose incorporated in meal) to assess hepatic and peripheral glucose metabolism. Following vildagliptin, suppression of endogenous glucose production (EGP) during 6-h MTT was greater than with PBO (1.02±0.06 vs 0.74±0.06 mg/kg•min, p=0.004) while rate of total glucose disposal (TGD) was similar. During 6-h MTT performed with vildagliptin, insulin secretion rate (ISR)-calculated by deconvolution of C-peptide plasma concentration-increased by 21% (p=0.003 vs PBO) despite significant reduction in mean plasma glucose (213±4 vs 230±4 mg/dl, p=0.006); consequently, the insulinogeneic index (ΔISR÷Δplasma glucose) increased by 29% (p=0.01). Suppression of mean plasma glucagon during MTT was 5 times greater with vildagliptin (p<0.02). From 6PM to 8AM EGP was significantly reduced (0.8±0.06 vs 0.5±0.06 mg/kg•min, p<0.003) in vildagliptin vs PBO and the decline in EGP was positively correlated in the treated arm (r=0.55, p<0.03) with the 14 mg/dl decrease in FPG observed at 8AM.

Conclusion: During a MTT, vildagliptin augments insulin secretion and inhibits glucagon release, leading to enhanced suppression of EGP. During the entire overnight period (6PM–8AM), a single dose of vildagliptin reduced FPG by enhancing suppression of EGP.

Supported by Novartis Pharmaceuticals

0042

Sustained efficacy and tolerability of vildagliptin monotherapy during one-year treatment of drug-naive patients with type 2 diabetes

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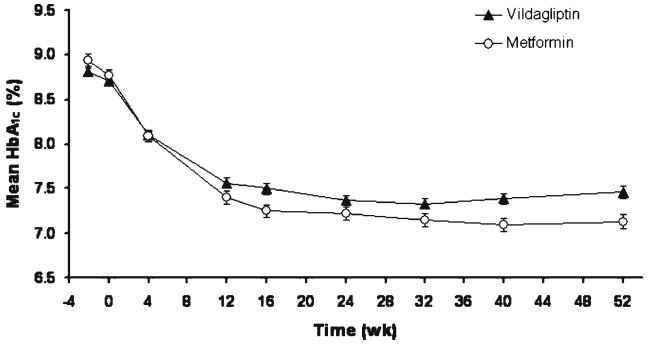
Background and Aims: Vildagliptin is a potent and selective DPP-4 inhibitor that improves glycemic control by increasing pancreatic islet responsiveness to glucose. The purpose of the present study was to assess the efficacy and tolerability of vildagliptin in comparison to metformin in drug-naïve patients (pts) with type 2 diabetes (T2DM).
Methods: This was a 52-wk, multicenter, randomized, double-blind, active-controlled, parallel-group study of vildagliptin 50 mg bid (n=526) and metformin, titrated to 1000 mg bid (n=254) in drug-naïve pts with T2DM. The primary endpoint was the between-group difference and the associated 95% confidence interval in the adjusted mean change (AMΔ) in HbA_{1c} from baseline (BL) to Wk 52 or study endpoint.
Results: The demographic and disease characteristics of the two groups were well-balanced at BL. In the randomized population, mean age=53.1 y, BMI=32.4 kg/m^2, HbA_{1c}=8.7% and disease duration=2.4 y. As shown below, both vildagliptin 50 mg bid and metformin 1000 mg bid rapidly decreased HbA_{1c} and this improvement was sustained throughout 52-wk treatment. The AMΔ from BL to endpoint was −1.0±0.1% in pts receiving vildagliptin and −1.4±0.1% in those receiving metformin. The study did not establish non-inferiority of vildagliptin 100 mg daily to metformin 2000 mg daily in these drug-naïve patients.



However, the percentage of pts reporting side effects was 70.1% in the vildagliptin group and 75.4% in the metformin group, and the incidence of gastrointestinal (GI) side effects was significantly less in patients pts receiving vildagliptin (21.8%) vs those receiving metformin (43.7%, P<0.001). Diarrhea was reported by 6.0% vs 26.2% of pts treated with vildagliptin vs metformin; nausea, by 3.3% vs 10.3%; abdominal pain, by 2.3 vs 7.1%; dyspepsia, by 1.2 vs 4.8%; vomiting, by 2.1 vs 4.4% and flatulence, by 1.0 vs 4.0%. Further, a smaller percentage of pts in the vildagliptin group (4.2%) discontinued due to an adverse event than in the metformin group (7.1%). A mild hypoglycemic episode was reported by 3 vildagliptin-treated pts (0.6%) and by one metformin-treated pt (0.4%). Vildagliptin did not cause weight gain (AMΔ body weight=0.3±0.2 kg) despite the improvement in metabolic control.
Conclusions: 1) a clinically meaningful reduction in HbA_{1c} is sustained throughout one-year treatment with vildagliptin 50 mg bid, 2) vildagliptin is well-tolerated and associated with fewer GI side effects than metformin.
Supported by Novartis

OP 8

Predictors and markers of cardiovascular disease and mortality

0043

Predictors of mortality in type 1 diabetes — a 20 year prosepective study

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Background and Aims: To evaluate the prognostic significance of microalbuminuria and overt diabetic nephropathy and other putative risk factors for all-cause mortality in type 1 diabetic patients in a 20 year prospective observational follow up study.
Materials and Methods: All adult type 1 diabetic patients, duration of diabetes ≥5 years, attending our clinic were identified in 1984 (n=939); At baseline 593 patients had normal urinary albumin excretion (≤30 mg/24 h), 181 had persistent microalbuminuria (>30 and <300 mg/24 h), and 165 had overt nephropathy (≥300 mg/24 h).
Results: After 20 years of follow up 34% of patients with normoalbuminuria, 47% of patients with microalbuminuria (p<0.001 vs. normoalbuminuria) and 62% of patients with overt nephropathy at baseline died (p<0.001). A stepwise multiple Cox regression analysis revealed the following predictors of all-cause mortality: male sex 1.84 (1.45 to 2.34) (relative risk (95% confidence interval)), age (years) 1.05 (1.04 to 1.06), smoking 1.67 (1.31 to 2.12), social class 4 (compared to class 5) 0.57 (0.44 to 0.75), Log_{10} urinary albumin excretion rate 1.50 (1.29 to 1.74), Cockroft–Gault estimated creatinine clearance (ml/min) 0.99 (0.986 to 0.995) and haemoglobin A_{1c} (%) 1.7 (1.01

to 1.14), systolic blood pressure (mm Hg) 1.01 (1.01 to 1.02), vibration perception threshold (mV) 1.01 (1.009 to 1.02). Height, presence of retinopathy, pulse pressure, diastolic blood pressure, and duration of diabetes were not included in the model. Median survival time after onset of overt diabetic nephropathy was 13.9 (11.8 to 17.2) years.
Conclusion: Abnormally elevated urinary albumin excretion and other potentially modifiable risk factors such as hypertension, smoking, poor glycaemic control and social class predicts increased mortality in type 1 diabetic patients.

0044

High levels of advanced glycation end products predict increased cardiovascular disease mortality in women, but not in men with type 2 diabetes. A population-based 18-year follow-up study

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Background: Advanced glycation end products (AGEs), short- and long-term modification products of glycation or glycoxidation of proteins and lipids, have been linked to the development of premature atherosclerosis in non-diabetic and diabetic subjects. We have recently reported that high serum levels of AGEs predict increased coronary heart disease (CHD) mortality in nondiabetic women, but not in nondiabetic men. The present study investigated whether cardiovascular disease mortality (CVD) was predicted by increased serum levels of AGEs in patients with type 2 diabetes in a population based study.
Methods and Results: Serum levels of AGEs were measured with a competitive immunoassay utilizing a polyclonal anti-AGE antibody and time delayed fluorescence (DELFIA), in samples obtained at baseline examination of a random sample of 874 individuals (488 men and 386 women) with diabetes aged 45 to 64 years living in Kuopio, East Finland or Turku, West Finland in 1982 to 1984. After 18 years of follow-up, all-cause mortality, CVD, and CHD mortality were registered on the basis of death certificates. Multivariate Cox regression model showed a significant association of serum AGEs with all-cause (P=0.004), and CVD mortality (P=0.042) in women, but not in men. Fasting serum AGEs in the highest quartile was an independent risk factor for all-cause (hazards ratio [HR], 1.61; 95% CI, 1.22 to 2.12; P=0.001), and CVD mortality (HR 1.42; 95% CI 1.01 to 2.00; P=0.046) in women, even after adjusting for confounding factors including highly sensitive C-reactive protein (Table). Non-CVD deaths were mainly due to diabetes or cancer.
Conclusions: The present study is the first to show that serum levels of AGEs predict total and CVD mortality in women with type 2 diabetes.

	Model 1		Model 2	
p-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Total mortality				
All	1.14 (0.95–1.36)	0.160	1.18 (0.98–1.43)	0.077
Men	0.85 (0.66–1.09)	0.197	0.89 (0.69–1.16)	0.399
Women	1.61 (1.24–2.11)	<0.001	1.61 (1.22–2.12)	0.001
Non-CVD mortality				
All	1.05 (0.76–1.45)	0.757	1.16 (0.83–1.63)	0.382
Men	0.66 (0.41–1.07)	0.091	0.71 (0.42–1.19)	0.189
Women	1.99 (1.27–3.13)	0.003	2.11 (1.32–3.37)	0.002
CVD mortality				
All	1.18 (0.95–1.46)	0.141	1.19 (0.95–1.49)	0.129
Men	0.94 (0.70–1.26)	0.678	0.98 (0.72–1.32)	0.876
Women	1.45 (1.04–2.02)	0.027	1.42 (1.01–2.00)	0.046
CHD mortality				
All	1.13 (0.87–1.45)	0.361	1.14 (0.87–1.49)	0.332
Men	0.85 (0.61–1.19)	0.352	0.87 (0.62–1.24)	0.449
Women	1.48 (0.99–2.22)	0.055	1.51 (1.00–2.29)	0.052

High serum levels (the gender-specific highest quartile range vs. the three lowest quartile ranges: cutpoints ≥10.3 mU/l in diabetic men and 9.6 ≥mU/l in diabetic women) of AGEs as a risk factor for total, non-CVD, CVD and CHD mortality in diabetic subjects. Adjusted in model 1: for age, area of residence, and area of residence (in all), total cholesterol, current smoking, hypertension, body mass index, HDL cholesterol, triglycerides, and postmenopausal status (in women); in model 2: as in model 1 and additionally for CRP.

Supported by the Norwegian Foundation for Health and Rehabilitation, Aker Diabetes Research Fund

0045

Skin autofluorescence is a better predictor for mortality than HbA1c in well-controlled type 2 diabetes patients

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Background and Aims: In DCCT substudies in type 1 diabetes skin Advanced Glycation End products (AGEs) predicted diabetic complications better than HbA1c. Type 2 diabetes mellitus is also associated with increased formation of AGEs. Some AGEs encompass a characteristic fluorescence pattern, which can be non-invasively measured as skin autofluorescence (AF). We have previously demonstrated that skin AF is elevated in type 2 diabetes mellitus, and strongly related to, and also predictive of chronic complications. This study aims to assess whether skin AF predicts life expectancy of T2DM-patients.
Materials and Methods: In 2001, 973 primary care T2DM-patients, participating in a shared-care project, were included. This group (mean age 66 yrs) was well-controlled (mean HbA1c 7.0%) and had a relatively short diabetes duration (median 4.2 yrs), 19% were current smokers. Skin AF was assessed at that time, and is calculated by dividing the mean value of the emitted light intensity per nm between 420–600 nm by the mean value of the excitation light intensity per nm between 300–420 nm, using a prototype of the current AGE reader.Vital status was assessed at January 2005. Life expectancy was assessed by transforming survival time to standardised survival time (SST) allowing adjustment for the baseline mortality rate of the general population.
Results: During a median follow-up of 3.2 years, 86 patients died (44 from cardiovascular disease). All cause and cardiovascular mortality were increased in the non-smoking group, with a relative risk (RR) of 2.4 (CI 1.3–3.9; $p<0.005$) and 2.7(1,2–6.5; $p=0.02$), respectively. This was not found in the smokers (19%). In a Cox regression analysis smoking, the presence of peripheral vascular disease and skin AF were the only 3 remaining factors for mortality or lifetime expectancy. HbA1c (actual, or of the previous year), blood pressure and lipid parameters were not.
Conclusions: This study shows that skin AF is a strong and independent predictor of mortality and life expectancy in a non-smoking and well-supported T2DM population, and performs better as predictor than conventional measures like HbA1c, and blood pressure. This makes it a promising tool for rapid, non-invasive cardiovascular risk assessment in type 2 diabetes mellitus.
Supported by Diabetes Fonds Nederland

0046

High level Impaired Fasting Glucose (IFG) (110–126 mg/dl), but not low level IFG (100–110 mg/dl) is associated with increased mortality and heart failure after MI

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Background and Aims: In a previous study, we have shown that patients hospitalized for acute MI, with IFG, according to the previous ADA criteria (110–126 mg/dl) had an overall worse outcome characterized by a significant higher risk of developing heart failure during their hospital stay. Since 2004, ADA has defined new criteria for IFG (fasting blood glucose between 100 and 126 mg/dl), because of evidence for increased cardiovascular risk in individuals with fasting glycemia above 100 mg/dl. However, so far the short-term prognosis after MI according to the new ADA criteria for IFG, and more particularly for the lower IFG level (100–110 mg/dl) has not been evaluated. This prompted us to study in-hospital outcomes according to the new criteria for IFG, using a large French regional MI registry collecting data from patients hospitalized for acute MI.
Materials and Methods: We studied 2353 patients with acute MI. The diagnosis of fasting glucose abnormality was performed using the mean of the fasting glycemias obtained at day 4 and 5 after admission. Patients were classified as diabetics (D) (known diabetes or FG≥126 mg/dl), high IFG (110≤FG <126 mg/dl), low IFG (100≤FG <110 mg/dl) and normal fasting glycemia (NFG) (FG <100 mg/dl).
Results: Among the 2353 patients, 968 (41.1%) were D, 262 (11.1%) were high IFG, 332 (14.1%) were low IFG and 791 (33.7%) were NFG. As compared to NFG individuals, in-hospital cardiovascular mortality was increased in high IFG subjects (5.3% vs 1.8%, $p=0.002$) but not in low IFG subjects (1% vs 1.8%). As compared to NFG individuals, in-hospital heart failure was increased in high IFG subjects (42% vs 20%, $p<0.0001$) but not in low IFG subjects (21% vs 20%). In multivariate analysis,after adjusment for age, gender, history of hypertension, anterior infaction, Killip class or LVEF, heart rate and blood pressure at admission, use of reperfusion therapy, high IFG, but not low IFG, was an independent factor for in-hospital mortality (OR: 2.33 [1.55–3.47], $p=0.03$) and for heart failure (OR: 1.70 [1.36–2.07], $p=0.01$).
Conclusion: The high level of the new ADA criteria for IFG (110–126 mg/dl) is an independent factor for in-hospital CV mortality and heart failure after MI, when the low level of the new ADA criteria for IFG (100–110 mg/dl) is not.

0047

NT-proBNP and mortality in type 1 diabetes

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Background and Aims: N-terminal pro brain natriuretic peptide (NT-proBNP) is a well-established prognostic biomarker in patients with heart failure and acute coronary syndrome. Recent data suggest that NT-proBNP may be an independent predictor of mortality in patients with diabetic nephropathy. We assessed whether NT-proBNP is a risk marker of death in patients with type 1 diabetes both with and without nephropathy.
Materials and Methods: Prospective cohort study in type 1 diabetes. Micro- and macro vascular complications were recorded at baseline, and NT-proBNP was measured in all patients. Five-year follow-up with recording of all-cause mortality.
Results: A total of 285 patients were included (median age 44, range 19–84 years, median duration of diabetes 19 years, range 3–56 years at baseline). Nineteen percent had hypertension, 7% had known cardiovascular disease (2% former stroke and 5% former myocardial infarction/angina), 9% had diabetic nephropathy and 18% had microalbuminuria. The five-year all-cause mortality rate was 6.0%.
At stepwise multivariate analysis, female gender ($p<0.0005$), high age ($p<0.0005$), presence of nephropathy ($p<0.05$), peripheral neuropathy ($p<0.01$) and former myocardial infarction/angina ($p=0.01$) were positively associated with elevated NT-proBNP at baseline.
All-cause mortality was significantly associated with high levels of NT-proBNP ($p<0.001$). After exclusion of subjects with known cardiovascular disease, hypertension and diabetic nephropathy (n=195) high NT-proBNP levels were still significantly associated ($p<0.0005$) with death.
Conclusion: Elevated NT-proBNP is associated with presence of microvascular complications as nephropathy and neuropathy. The present data suggest, that NT-proBNP is a novel strong risk marker of all-cause mortality in type 1 diabetic patients with and without overt nephropathy and independent of present cardiovascular disease.

0048

Insulin-like growth factor binding protein-1 predicts cardiovascular morbidity and mortality in patients with acute myocardial infarction and type 2 diabetes. A report from the DIGAMI 2 trial

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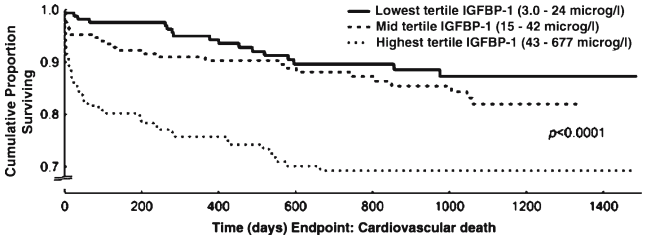
Background and Aims: Although not a consistent finding there are indications that high levels of insulin-like growth factor binding protein-1 (IGFBP-1) may be related to cardiovascular (CV) mortality and morbidity. The aim of this study was to test the hypothesis that high levels of IGFBP-1 predict future CV mortality and morbidity in patients with acute myocardial infarction (AMI) and type 2 diabetes (T2DM).
Materials and Methods: The DIGAMI 2 trial recruited 1253 patients with T2DM and suspected AMI of which 575 patients (men 67%), the present material, were enrolled in a biochemical program with repeated blood sampling. Primary and secondary endpoints were CV mortality and a composite of CV events (CV death, reinfarction or stroke). Biochemical and clinical factors at admission where analyzed by a multiple Cox proportional hazard regression analysis. The results are presented as HR and 95% CI.
Results: During a median follow-up of 2.2 years, 102 out of the 575 patients (18%) died from CV reasons while 175 (30%) suffered from a CV event. Age (+5 years; 1.6, 1.4–1.8; $p<0.001$), Ln S-IGFBP-1 (1.5, 1.2–1.9; $p<0.001$) and Ln S-Creatinine (2.0, 1.2–3.5; $p=0.013$) remained independent predictors of CV mortality. The model predicting major CV events contained the same variables and in addition previous congestive heart failure (1.5, 1.1–2.1; $p=0.025$). Variables that were eliminated in best subset analyses were B-glucose, HbA1c, S-Potassium, S-cholesterol, S-triglycerides, BMI, gender, duration of diabetes, smoking, previous AMI and U-albumin. Figure is presenting Kaplan–Meier curves of CV mortality statified for IGFBP-1 tertiles ($p<0.001$).
Conclusion: High levels of IGFBP-1 in the early phase of an AMI indicate increased risk for CV mortality and morbidity in patients with T2DM.



OP 9

Retinopathy

0049

Early treatment with combined antisense oligonucleotides against ECM components prevents vascular BM thickening and lesions in diabetic rat retinas

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Background and Aims: Thickening of capillary basement membrane (BM) has been widely observed in diabetic humans and animals, and is the most prominent histological abnormality in the diabetic retina. Studies have shown that diabetes increases BM gene expression in retinal vascular cells and that the excess synthesis of BM components contributes, at least in part, to the development of thickened vascular BMs. Our findings suggest that combined antisense oligos administered soon after diabetes inhibit abnormal synthesis of extracellular matrix (ECM) components and reduce pericyte loss, acellular capillaries, and vascular leakage in the diabetic retina. In this study we determined whether combined antisense oligos approach is effective if administered *after* 3 months of diabetes in preventing vascular lesions associated with diabetic retinopathy.
Materials and Methods: Streptozotocin-induced diabetic rats were randomly assigned to receive either combined AS-oligos (fibronectin (FN), collagen IV (Col), and laminin (LM) oligos) or random (Ran) oligos. After 3 months of diabetes, 3 uM combined AS oligos or Ran oligos were administered monthly for 3 months by intravitreal injection. At the end of 6 month period, the rats were sacrificed, glycohemoglobin level measured, retinas isolated, retinal trypsin digest (RTD) prepared and Western Blot (WB) analysis performed for FN, Col, and LM protein expression. Retinal vascular BM thickening was assessed by electron microscopy, and pericyte loss/acellular capillaries were determined from RTD preparations stained with hematoxylin and PAS.
Results: WB analysis showed retinal FN, Col, and LM level was upregulated in the diabetic rats compared to those of non-diabetic rats (228±25%, 178±21%, 155±18% of control, respectively, $p<0.05$). The eyes injected with combined antisense oligos showed reduced protein expression for the three components (131±11% of control, 136±28% of control, 114±17% of control, respectively, $p<0.05$). Retinal capillary BM thickness measured in diabetic rats showed a significant increase compared to those of non-diabetic control rats (148±19 nm vs. 91±15 nm, $p<0.01$). The BM width in retinal capillaries of diabetic rats injected with combined antisense oligos showed a significant reduction compared to those of untreated diabetic rat eyes (109±21 vs. 148±19 nm, $p<0.02$). Diabetic rats treated with Ran oligos showed no difference in the retinal capillary width. The number of pericyte loss and acellular capillaries was significantly increased in retinas of diabetic rats compared with non-diabetic control rats (160% of control, $p<0.03$, and 240% of control, $p<0.02$, respectively). Diabetic rats treated with combined AS oligos exhibited a significant decrease in the number of pericyte loss and acellular capillaries compared to those of untreated diabetic rats (160% vs. 110% of control, $p<0.01$, and 240% vs. 160% of control, $p<0.01$, respectively. The number of pericyte loss and acellular vessels of diabetic rats that received random oligos were not different from those of untreated diabetic rats. Glycohemoglobin levels were elevated in all diabetic rats compared to those of control rats (1.7±0.3 fold).
Conclusion: Early treatment with combined (FN, Col, LM) antisense oligos may provide a beneficial effect in reducing BM thickening and vascular lesions associated with diabetic retinopathy.
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0050

WITHDRAWN

0051

Downregulation of high glucose-induced fibronectin overexpression modulates integrin expression in microvascular endothelial cells

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Background and Aims: Vascular basement membrane thickening and retinal vascular cell loss are two prominent lesions associated with diabetic retinopathy. While increased synthesis of extracellular matrix (ECM) compo-

nents has been shown to contribute to the development of basement membrane thickening, the underlying cause of retinal vascular cell loss in diabetic retinopathy remains unclear. Studies have shown that high glucose and diabetes increase integrin expression, including fibronectin (FN) specific integrin receptor alpha 5 beta 1, and may compromise interactions between cells and their basement membranes. Having downregulated fibronectin overexpression with antisense oligonucleotides targeted to fibronectin transcript, in this study, we have determined whether direct modulation of fibronectin expression alters expression of integrin alpha 5 subunit.

Materials and Methods: To determine whether modulation of FN expression affects alpha 5 subunit expression, rat microvascular endothelial cells (RMECs) were grown in high glucose condition for 9 days and transfected with 0.2 uM FN–AS oligos. After 3 days post-transfection, cells were harvested and protein analyzed by Western blot for FN and alpha 5 subunit expression. In parallel, cells plated on cover slips and similarly transfected with FN-AS oligos, FN and alpha 5 immunoreactivity were examined under fluorescence microscopy. Cells transfected with random oligos were used as control in Western blot analysis and experiments using immunofluorescence microscopy.

Results: Western blot analysis showed increased alpha 5 expression in cells grown in HG condition compared to cells grown in N medium (233±23% of control, $p<0.01$). In cells transfected with AS-FN oligos, alpha 5 subunit expression was significantly reduced (161±30% of control, $p<0.01$) compared to cells grown in HG condition. The cells grown in HG medium and transfected with random oligos showed no effect in either alpha 5 subunit or FN expression. Immunofluorescence microscopy indicated increased integrin alpha 5 immunostaining in RMECs grown in HG medium compared to cells grown in N medium (140±11% of control, $p<0.01$). The alpha 5 subunit expression was reduced (101±10% of control, $p<0.05$) in cells transfected with AS-FN oligos compared to cells grown in HG condition. The cells grown in HG medium and transfected with random oligos showed no effect. In both analyses, strong correlations were observed between FN expression and integrin expression.

Conclusion: The findings indicate a direct link between FN and integrin expression in microvascular endothelial cells. While downregulation of FN overexpression may prevent basement membrane thickening, it is possible that it may also prevent integrin overexpression associated with endothelial dysfunction in diabetes.

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0052

Tight blood pressure control prevents early inflammatory events in diabetic retina

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Background and Aims: Hypertension is the main secondary factor associated with diabetic retinopathy (DR). The mechanisms by which hypertension increase the risk for DR are poorly understood. The aims of this study were to investigate the effect of diabetes and hypertension on early inflammatory phenomena in the retina of hypertensive and diabetic rats and to test the effects of blood pressure control on retinal inflammation.

Materials and Methods: Four week old spontaneously hypertensive rats (SHR) and their normotensive counterpart Wystar Kyoto (WKY) rats were rendered diabetic by intravenous injection of streptozotocin. Control groups received only vehicle (Wc and Sc). Diabetic SHR rats (Sd) were randomized for no antihypertensive treatment, for treatment with losartan (Sd+Los), or triple therapy (Sd+Tri). After 20 days, rats were sacrificed and the retinas were collected.

Results: The number of immunohistochemically detected ED1/microglial positive cells (13.4±2.8 vs 25.0±0.07, $p<0.0001$) and the expression of ICAM-1 (0.7±0.1 vs 1.6±0.6 arbitrary units, $p<0.004$) in the retina were significantly higher in diabetic SHR than in the control. The NF-kB p65 levels were higher in SHR compared with WKY groups (0.4±0.2 vs 1.6±0.6 arbitrary units, $p<0.005$) and its increment in diabetic SHR was not significant. These abnormalities were not observed in diabetic WKY rats. The control of hypertension in SHR, either with triple therapy or losartan, reestablished the microglial status (4.5±1.6 and 5.0±1.0 vs 6.6±2.0 for Sd+tri and Sd+los vs Wc, $p=0.1$), ICAM-1 retinal levels (0.5±0.2 and 0.4±0.2 vs 0.3±0.1 arbitrary units for Sd+Tri and Sd+Los vs Wc, $p=0.1$), and NF-kB p65 levels (0.7±0.1 and 0.5±0.06 vs 0.4±0.3 arbitrary units for Sd+Tri and Sd+Los vs Wc, $p=0.1$) to WKY levels.

Conclusion: The concomitance of diabetes and hypertension leads to exuberant inflammatory response in the retina and the tight control of blood pressure was able to abrogate these abnormalities. Inflammatory abnormalities may be involved in the mechanism by which hypertension exacerbates retinopathy in patients with diabetes.

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0053

Down-regulation of the anti-angiogenic factor PEDF by insulin, rosiglitazone and VEGF: implications for neovascularization in poorly controlled diabetes

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Background and Aims: Pigment Epithelium Derived Factor (PEDF) is an important anti-angiogenic protein associated inversely with diabetes-related microvascular complications, especially retinopathy, but very little is known about the metabolic and pharmacological regulation of PEDF expression in vascular cells.
Materials and Methods: Human umbilical smooth muscle cells were cultured to confluence, then starved and quiesced in low-serum medium for 24 h. Cells were then exposed to VEGF 10 ng/mL, insulin 400 nM, rosiglitazone 100 μM, and D-glucose 10–20 μM for 24 h. Following cell lysis, RNA was extracted for real time RT–PCR in Bio–Rad iCycle PCR programmes.
Results: In comparison with untreated cells, three experimental interventions produced significant decreases in PEDF mRNA expression relative to the housekeeping gene B2M: e.g VEGF 0.672-fold reduction in expression (n=4, p=0.018), rosiglitazone 0.598-fold (n=4, p<0.01) and insulin 0.123-fold (n=4, p<0.01). In contrast, D-glucose had no effect (1.08-fold expression; n=14, p=0.574).
Conclusion: This is the first study to show that rosiglitazone, VEGF and especially insulin down-regulate the expression of an important molecule, PEDF, which inhibits key steps in angiogenesis.

0054

The apoptotic response of retinal pericytes after fluctuation from high to low glucose: a comparison between bovine and human models

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Background and Aims: Loss of pericytes is one of the earliest events in diabetic retinopathy, due to mechanisms that have not yet been elucidated. The pathophysiological role of pericytes has been investigated, so far, using primary bovine or rat cells in in vitro studies. However, some observations suggest that human and bovine pericytes may behave differently in experimental conditions designed to mimick the diabetic milieu. This study aimed at verifying whether pulsed exposure to high glucose may have different effects on human (HRP) and bovine retinal pericyte (BRP) apoptosis.
Materials and Methods: BRP and HRP were grown in physiological (5.6 mmol/L) or high (28 mmol/L) glucose for 7 days, and then exposed to glucose 5.6 mmol/L for another 24, 48 or 72 h. DNA fragmentation was evaluated by ELISA, as a marker of early apoptosis. In parallel, the expression of two genes involved in apoptosis, *bax* and *p53,* was determined by RT–PCR. Results are expressed as percentages of BPR grown in physiological glucose.
Results: Apoptosis rate in physiological glucose in HRP was 9.3% than in BRP (p=0.000). Seven-day exposure to high glucose increased apoptosis in BRP (190.5±77.0%, p=0.050), but not in HRP (16.6±22.7%). However, when BRP kept in high glucose were returned to 5.6 mmol/L, apoptosis reverted within 24 hrs to the levels measured in BRP that had always been in physiological glucose. On the contrary, in HRP kept for 7 days in high glucose, apoptosis increased 48 (p=0.010) and 72 hrs (p=0.010) after re-exposure to 5.6 mmol/L.
bax expression increased and decreased concurrently with DNA fragmentation in BRP and HRP, with a clear over-expression in HRP within 24 h of returning to physiological glucose. *p53* mRNA was unchanged in both cell types, regardless of glucose exposure.
Conclusion: Human pericytes appear to be less prone to apoptosis induced by persistently high glucose than bovine cells. However, while BRP recover when brought back to a physiological milieu, HRP appear to be more vulnerable to downwardly fluctuating glucose levels. These data reinforce the hypothesis that daily fluctuations in blood glucose play a major role in the development of diabetic retinopathy.

OP 10

Novel genes in type 2 diabetes

0055

N-myc downstream-regulated gene 2 is a novel type 2 diabetes candidate gene

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Background and Aims: Skeletal muscle is the major site for insulin-mediated glucose uptake and it is therefore a therapeutic target to reduce blood glucose levels in insulin

resistant and diabetic individuals. In *Psammomys obesus (P. obesus)*, a polygenic rodent model of obesity and type 2 diabetes, we used microarray analysis to identify genes in the red gastrocnemius muscle that responded to exercise training. We identified a number of exercise-responsive genes, including N-myc down-regulating gene 2 (NDRG2). In both rodents and humans, NDRG2 is predominantly expressed in the brain and skeletal muscle. In skeletal muscle NDRG2 is a substrate of AKT/PKB, SGK1, RSK, and p70S6K but its function is not known. To further characterise NDRG2 and its possible role in diabetes, NDRG2 gene expression was analysed in normal and insulin resistant *P.obesus* and human individuals and in differentiating myoblasts. In addition, a yeast two hybrid screen of a human skeletal muscle cDNA library was performed using NDRG2 as bait to identify novel interacting protein partners of NDRG2.

Materials and Methods: Normal glucose tolerant (NGT) and impaired glucose tolerant (IGT) *P.obesus* (n=8 for each) were fasted for 24 h. NGT (n=8) and IGT (n=20) human subjects were fasted for 8 hours. NDRG2 mRNA levels were quantitated by real time PCR and statistical analysis was performed using independent samples t-test. For the yeast two hybrid screen, full length NDRG2 cDNA along with a human skeletal muscle cDNA library was transformed into MaV203 yeast cells. The HIS3, URA3 and lacZ reporter genes were utilised as selectable markers of protein interaction. Protein interactions were confirmed following co-immunoprecipitation studies of over-expressed NDRG2, Arfaptin 2 and Protein interacting with C kinase 1 (PICK1) in HEK293 cells.

Results: NDRG2 gene expression was increased significantly in skeletal muscle from human IGT subjects (P=0.005) and from IGT *P.obesus* (P=0.036) compared to NGT controls. In differentiating L6 myotubes, NDRG2 gene expression was up-regulated 13 fold. The yeast two hybrid screen identified 9 clones that strongly interacted with NDRG2. Eight clones were identified as Arfaptin 2, a protein known to interact with RAC and ADP-ribosylation factors (ARFs) GTPases, molecules that are linked to the control of the cytoskeleton and membrane trafficking, respectively. The remaining clone was found to be PICK1, a protein that binds lipids and regulates protein traffic. Both Arfaptin 2 and PICK1 contain an arfaptin homology domain indicative of proteins involved in intracellular transport. These interactions were confirmed by co-immunoprecipitation analyses in HEK293 cells.

Conclusion: Here, we have identified NDRG2 as a novel interacting partner of PICK1 and Arfaptin 2 revealing for the first time a potential role for this molecule. Since NDRG2 is a substrate of kinases integral to insulin signaling and found differentially expressed in IGT *P. obesus* animals and human individuals, together these data suggest an involvement for NDRG2 in protein trafficking and in the development of type 2 diabetes.

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0056

The H566H polymorphism of the lamin A/C gene (*LMNA*) on chromosome 1q associates with type 2 diabetes and elevated fasting glycaemia in studies of 7,098 Danish whites

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Background and Aims: Evidence for linkage of type 2 diabetes to a region on chromosome 1q21–q24 has been provided in several populations. Among genes in this region is *LMNA* encoding nuclear lamin A and C proteins which serve as nuclear lamina intermediate-filament proteins in nearly all somatic cells. Rare mutations in *LMNA* cause several distinct monogenic syndromes including different subtypes of muscular dystrophy and familial partial lipodystrophy; the latter characterized by altered fat distribution, insulin resistance, and type 2 diabetes. A common synonymous variant (H566H) in *LMNA* has previously been associated with features of the metabolic syndrome. Thus, the aim of the present study was to examine the H566H variant in relation to type 2 diabetes and associated quantitative metabolic traits in Danish whites.

Materials and Methods: The *LMNA* H566H variant (c. 1908C>T, rs4641) was genotyped in the population-based Inter99 cohort (n=5,754) and in a group of 1,324 type 2 diabetic patients using mass-spectrometry of PCR products.

Results: The minor T-allele was more prevalent among 1,324 type 2 diabetic patients than in 4,386 subjects with normal glucose tolerance (27.0% [95% CI: 25.3–28.7%] vs. 24.5% [23.6–25.4%]; odds ratio 1.17 [1.03–1.26]; p=0.01). Adjusting for the effect of age, gender, and BMI strengthened the association (additive model: p=0.003). Furthermore, a study of quantitative traits among 5,395 population-based sampled, middle-aged people without known diabetes revealed that individuals carrying the minor T-allele had higher fasting plasma glucose concentration (CC: mean±SD 5.52±0.75 mmol/l, CT: 5.56±0.87, TT: 5.60±1.0; additive model: p=0.008). No association with obesity or measures of insulin resistance was observed.

Conclusion: The minor T-allele of the synonymous H566H variant in *LMNA* associates with type 2 diabetes and elevated fasting glycaemia in the general population. A possible mechanism of altered protein function is through a change in lamin A to C ratio since the nucleotide substitution occurs at the site of alternative mRNA splicing in exon 10.

0057

The genetic variation in the gene (*TNMD*) is associated with impaired glucose metabolism and conversion to type 2 diabetes in men of the Finnish Diabetes Prevention Study

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Background and Aims: We recently demonstrated that long-term weight reduction changes the gene expression profile of adipose tissue in overweight individuals with impaired glucose tolerance. One of the responding genes was the X-chromosomal tenomodulin (*TNMD*), whose expression was decreased by weight reduction. Tenomodulin is a transmembrane protein expressed mainly in extracellular matrix of tendons, ligaments and eyes. In this study our aim was to study genetic associations between *TNMD* SNPs, glucose metabolism and the risk for T2DM in the Finnish Diabetes Prevention Study (DPS).
Methods: The DPS is a longitudinal intervention study consistiong of 522 obese or overweight subjects with impaired glucose tolerance (IGT). The seven SNPs from two haploblocks of the *TNM*D gene were selected for association studies on the basis of HapMap data. Genotyping was performed by Taqman allelic discrimination method. The linkage disequilibrium statistics were analyzed by Haploview software. Associations of SNPs with glucose and insulin levels were analyzed by general linear model using univariate analysis and the associations of SNPs with the progression of IGT to T2DM by Cox regression using SPSS 11.5 software.
Results: Genotypic effects of *TNMD* gene SNPs were gender-specific. The two SNPs, rs2073163 and rs1155794 belonging to the same haploblock were associated with 2-hour glucose levels in men (p=0.038 and 0.011). Furthermore, the same two markers and an additional marker, rs2073162 associated with the conversion of IGT to T2DM in men. The genotypes associating with elevated 2-hour glucose values were approximately two times more likely to develop T2DM during follow-up. The hazard ratios were 2.192 (p=0.025) for rs2073162, 2.191 (p=0.027) for rs2073163 and 1.998 (p=0.054) for rs1155974, with the model adjusted for BMI, WHR, fasting plasma glucose and intervention.
Conclusion: These results suggest that SNPs of the *TNMD* gene mediate gender-dependent effects on insulin and glucose metabolism and on conversion of IGT to T2DM in men. Furthermore, this study provides evidence that extracellular matrix components may play a role in the development of T2DM via modulating glucose metabolism.
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0058

The TT genotype of the subtype 2 bradykinin receptor (BDKRB2) is associated with a high risk for type 2 diabetes (T2D) in the general population: the DESIR study

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Background and Aims: The Kallikrein-Kinin system may affect glucose metabolism through an insulin facilitating action, as supported by experimental data and the prevention of diabetes by ACE inhibitors in cardiovascular trials. The bradykinin metabolism and effectiveness are conditioned by subtype 2 receptor (BDKRB2), whose C-58T polymorphism was reported to affect blood pressure, cardiovascular risk and diabetic nephropathy. We tested this polymorphism on risk for T2D in a large cohort from the French general population.
Materials and Methods: Subjects took part in DESIR (Epidemiologic Data on the Insulin Resistance Syndrome), a cohort of 2576 men and 2636 women from the middle-aged general population who were clinically and biologically evaluated at inclusion and at 3, 6 and 9-year visits. We genotyped them for the C-58T BDKRB2 promoter polymorphism. We studied the association of this polymorphism with the prevalence of diabetes at inclusion and its incidence during the 9-year follow-up. We used a logistic model for the incidence of diabetes taking into account the BMI, age, fasting glycemia at baseline, waist circumference and gender. Also, we tested the impact of the genotypes on insulin resistance.

Results: The C and T allelic frequencies were 57 and 43% respectively. The distribution of genotypes was in Hardy–Weinberg equilibrium. The genotype frequencies differed according to the presence or not of diabetes at baseline (Table, Prevalence: p=0.002) and the incidence of new cases of diabetes during the follow-up (Table, Incidence: p=0.01), consistent with a recessive effect of the T allele.

Prevalence of diabetes at baseline	CC	CT	TT	Total
No Diabetes	1592 (97.3%)	2373 (97.9%)	868 (95.3%)	4833 (100%)
Diabetes	45 (2.7%)	50 (2.1%)	43 (4.7%)	138 (100%)
Total	1637	2423	911	4971
Incidence of Diabetes during follow-up	**CC**	**CT**	**TT**	
No new-onset diabetes	1158 (95.7%)	1694 (95.5%)	621 (93.0%)	3473 (100%)
New-onset diabetes	52 (4.3%)	79 (4.5%)	47 (7.0%)	178 (100%)
Total	1210	1773	668	3651

The odds ratio of new-onset diabetes associated with the presence of the TT genotype was 1.77 (95% CI: 1.11–2.80) after adjustment. The population risk for incident T2D attributable to the BDKRB2 TT genotype was 10,3% (95% CI 2,0–18,0%). There was no difference in HOMA–IR according to genotypes.

Conclusions: These data suggest that the BDKRB2 C-58T polymorphism strongly affects the risk for T2D in the general population. This effect is not likely to be mediated by increased insulin resistance.

Supported by ALFEDIAM

0059

Variants in the *GPR40* gene and association with type 2 diabetes

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Background and Aims: The G protein-coupled receptor GPR40 is the first gene product identified to act as an extracellular membrane receptor for FFAs. The receptor is expressed mainly in pancreatic β-cells but also in the human brain. In β-cells *in vitro*, the receptor is activated by medium to long chain FFAs as well as by thiazolidinediones resulting in elevated Ca^{2+} concentrations and promotion of insulin secretion. These properties suggest that GPR40 could be a mediator of lipotoxicity and a potential candidate gene for Type 2 diabetes (T2D). We therefore studied whether variations in the *GPR40* locus are associated with T2D by re-sequencing the *GPR40* gene and genotyping single nucleotide polymorphisms (SNPs).

Materials and Methods: *GPR40* region was sequenced in 96 subjects to identify SNP polymorphisms and then SNP genotyping was used in 1929 patients with T2D and 754 healthy control subjects.

Results: This study suggests an effect of one polymorphism, located in a putative regulatory region of the GPR40 gene, on the risk of developing T2D (p=0.01). Furthermore, we observed an association with insulin secretion (p=0.01) and postprandial suppression of postprandial FFA levels (p=0.003) in a sample of 1011 healthy individuals from Botnia and the rest of Finland suggesting a role for GPR40 in the regulation of the lipotoxic effect of FFAs in humans.

Conclusion: In conclusion, variation in the GPR40 gene is associated with T2D, β-cell function and postprandial FFA levels.

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0060

Evidence that the *LARS2* gene represents a novel type 2 diabetes mellitus gene

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Background and Aims: It is known that mitochondrial dysfunction can lead to an increased risk for type2 diabetes mellitus (T2DM). *LARS2* encodes for the mitochondrial leucyl tRNA synthetase (LeuRS), which is essential for mitochondrial protein synthesis. Previously we have shown that an H324Q polymorphism associates with an enhanced risk for T2DM in populations from the Netherlands and Denmark. In this study we performed a replication study and reconstruct the *LARS2* LD structure for a haplotype based analysis.
Materials and Methods: *LARS2* SNPs were genotyped using Taqman SNP genotyping assays. Enzyme activity and mischarging rate were determined using recombinant enzymes. siRNA was performed using Rat-I and INS-1E cells.
Results: In this study the large UK Warren2 cohort was genotyped for the H324Q variant (OR 1.14 (95% CI 0.95-1.36), p=0.16, n=7063). In a meta analysis including almost 15000 subjects from five independent studies the overall OR was 1.23 (95% CI 1.06–1.41, p=0.004, n=14899). We have also reconstructed *LARS2* haplotypes in 1521 subjects from the Netherlands and Denmark. Eleven haplotype tagging SNPs were selected for further study based on data from the Hapmap database. We examined the LD structure and from this set five tagging SNPs were selected, which can discriminate between the most frequent haplotypes in our pilot study (n=354, f>2%). This set of SNPs was used to reconstruct the haplotypes of the larger cohort. None of the 7 common haplotypes observed were significantly associated with T2DM (P>0.05). When we added the H324Q variant to our analysis a single haplotype carrying the H324Q risk allele associated with T2DM susceptibility (P<0.05). Until now it is not clear via which pathogenic mechanism the H324Q variant enhances T2DM susceptibility. The enzyme activity of both wild type and mutant LeuRS was measured *in vitro*, but no significant differences were observed. Furthermore the mischarging rate was measured. Although not significant, the mischarging rate of the mutant enzyme was increased which will induce a higher error rate in mitochondrial protein synthesis. Because LeuRS is indispensable for mitochondrial protein synthesis we have tested whether or not mitochondrial protein synthesis is affected in fibroblasts of heterozygous H324Q carriers, but we found no significant differences. Preliminary siRNA data suggest that knockdown of LeuRS results, as expected, in a decreased function of mtDNA encoded proteins confirming the importance of correct tRNA synthetase activity for mitochondrial function.
Conclusion: In conclusion we have shown that the *LARS2* gene represents a T2DM susceptibility gene in several Caucasian populations. Additional risk or protecting haplotypes were not identified. Further research is needed to elucidate the exact pathogenic mechanism.
Supported by ZonMW

OP 11

Molecular components of the insulin secretion pathway

0061

Knockdown of the newly identified mitochondrial glutamate carrier GC1 and effects on glucose stimulated insulin secretion in INS-1E beta-cells

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Background and Aims: The mitochondrial glutamate carrier 1 (GC1), transporting glutamate with H+or in exchange to OH- through inner mitochondrial membrane, was previously cloned from human brain cDNA and showed high levels of expression in the whole pancreas. According to the role that glutamate might play in beta-cells as an additive factor in glucose stimulated insulin secretion, it was of interest to determine: i) putative expression of GC1 in insulin secreting cells, ii) in case of expression the intracellular localization of GC1, iii) whether knockdown of GC1 expression would affect glucose stimulated insulin secretion. Specifically, we cloned GC1 from the rat insulinoma INS-1E cells, generated an antibody against GC1, and investigated GC1 function with respect to insulin secretion by use of siRNA approach to specifically down-regulate GC1 in INS-1E beta-cells.
Materials and Methods: We first identified the rat GC1 ortholog in INS-1E beta-cells, which exhibits 95% similarity with its human counterpart. Next, we identified siRNA construct down-regulating GC1 by about 70%. This construct was cloned in a recombinant adenovirus and its down-regulation was assessed by immunoblotting using an antibody raised against GC1 selected sequence. The most efficient titer, i.e. 0.06 μl per well of 24-well plates, was used to transduce INS-1E cells for one hour and cells were subsequently analysed four days post-infection. As a control, an empty adenovirus construct was used. Following this transduction protocol, we measured several parameters, including mitochondrial membrane potential (rhodamine-123 fluorescence), cytosolic ATP changes (luciferase bioluminescence), cellular calcium levels (Fura-2 fluorescence). Insulin secretory responses (analysed by radioimmunoassay) to stimulatory glucose concentrations were tested with and without addition of cell permeant methylated glutamate.
Results: Immunofluorescence studies on INS-1E cells revealed expression and mitochondrial localization of GC1. Four days after infection with anti-GC1 siRNA adenovirus, down-regulation of GC1 expression was

observed by immunoblotting using the GC1 specific antiserum. In parallel experiment, the decrease in GC1 expression correlated with significant effects on insulin secretion. In control cells, elevation of glucose from basal 2.5 mM to 15 mM stimulated insulin secretion 3-fold. In cells transduced with the anti-GC1 siRNA construct, we observed inhibition of glucose evoked insulin secretion by 31% ($p<0.01$). The depletion of GC1, which promoted a decrease in insulin secretion, was partially restored upon provision of cell permeant methyl-glutamate. Parameters such as mitochondrial membrane potential, cytosolic ATP, and intracellular calcium levels, were not affected in cells treated with anti-GC1 siRNA construct compared to cells transduced with the empty construct. Cellular glutamate measurements are ongoing to determine changes in glutamate levels inside and outside of the mitochondrion, in order to provide insight into the directionality of glutamate transport across the mitochondrial membrane.

Conclusion: Insulin secreting cells express the newly identified glutamate carrier GC1. Knockdown experiments demonstrate that this mitochondrial carrier is necessary for the full development of the secretory response to glucose. Future investigations should delineate the specific role of GC1 in insulin release.

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0062

Presence and functionl importance of Kv1.1 channels in mouse islets: evidence from mice with truncated Kv1.1

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Background and Aims: Voltage-gated potassium channels (Kv channels) are involved in repolarization. In pancreatic beta cells blocking of Kv2.1 channels leads to increase in intracellular calcium and enhanced glucose-induced insulin secretion. However, the presence and functional importance of Kv1.1 channels in beta cells is presently unclear.

The megencephaly mouse BALB/cByJ-*Kv1.1*$^{mceph/mceph}$ (called mceph/mceph) displays neurological abnormalities, which are coupled to an 11 base-pair deletion in the gene coding for Kv1.1. The predicted MCEPH protein is truncated at amino acid 230 out of 495.

The aims of the present study were 1) to test for presence of Kv1.1 expression in pancreatic islets of normal mice and to verify abnormal expression in beta cells of mceph mice. 2) to test for functional importance of Kv1.1 on insulin secretion.

Materials and Methods: Mutant and wild type mice (BALB/cByJ) were used for experiments at the age of 2.5–3 months. Isolated islets of Langerhans were used for RT–PCR and histopathological analysis. Insulin release was measured in batch-type incubations and perifusion experiments.

Results: Weight, blood-glucose and histological staining of pancreata by hematoxylin/eosin did not differ between mutant and wild type mice. By RT–PCR we documented for the first time, expression of the Kv1.1 gene in normal mouse islets and its presence, in mutated form, in islets from the mceph/mceph mouse. Incremental glucose-induced insulin release was increased in mutant vs. wild type islets (70±10 vs. 51±7.4 μU/islet/h, $p<0.03$, n=11) in batch-type incubations. The Kv1.1 channel specific blocker dendrotoxin-k increased insulin secretion in wild type islets but had no effect in mutant islets tested both in batch type incubations and in a perifusion system (43% increase in wild type vs. 12% decrease in mutant islets, $p<0.05$, n=7).

Conclusion: Kv1.1 channels are present and of functional importance in mouse beta cells.

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0063

K_{ATP} channel independent coupling between insulin, membrane potential and $[Ca^{2+}]_c$ in mouse pancreatic β-cells

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Background and Aims: In pancreatic β-cells from wildtype (WT) mice hyperpolarization of the plasma membrane by insulin is usually ascribed to the opening of K_{ATP} channels. However, insulin alters the oscillations of membrane potential in K_{ATP} channel null, Sur1KO mice thus we investigated K_{ATP} channel independent mechanisms.

Materials and Methods: Membrane potential (V_m) recordings were performed with intracellular microelectrodes or in the perforated-patch configuration; $[Ca^{2+}]_c$ and $[Na^+]_c$ were determined using fluorescence techniques.

Results: In Sur1KO β-cells 200–400 nM insulin hyperpolarised V_m by −4±1 mV (n=4). An initial hyperpolarization of V_m was also induced by elevation of glucose from 0.5 to 15 mM (−8±1 mV; n=6). This effect coincided with a transient reduction of $[Ca^{2+}]_c$ (236±52 to 33±7 nM,

P<0.01; n=7) that was inhibited by the mitochondrial uncoupler FCCP (0.5 μM, n=6). The transient drop of $[Ca^{2+}]_c$ was not due to Ca^{2+} sequestration by the ER and persisted in the presence of the SERCA inhibitor cyclopiazonic acid (10 μM, n=7). Thus, the decrease in $[Ca^{2+}]_c$ may be caused by Na/Ca exchange. In WT β-cells glucose stimulation also resulted in a SERCA-independent transient reduction of $[Ca^{2+}]_c$ when K_{ATP} channels were blocked by 1 mM tolbutamide (278±28 to 51±10 nM, P<0.001; n=4). Interruption of insulin signaling by the tyrosine kinase inhibitor genistein (n=3) or the PI3 kinase blocker wortmannin (n=7) attenuated the Ca^{2+} lowering effect of glucose in Sur1KO β-cells significantly. The result suggested an autocrine effect of insulin. Since insulin is known to activate the Na/K ATPase we tested whether glucose stimulation reduces $[Na^+]_c$. Increasing glucose from 0.5 to 15 mM decreased $[Na^+]_c$ by 49±4 fluorescence units (n=3) potentially reflecting increased Na/K ATPase activity. The hyperpolarizing effect of glucose and the transient drop in $[Ca^{2+}]_c$ were completely suppressed by ouabain, a Na/K ATPase blocker (1 mM, n=3).

Conclusion: In Sur1KO and in tolbutamide-treated WT β-cells an increase in glucose concentration lowers $[Ca^{2+}]_c$ by activation of hyperpolarizing ionic currents. This effect is diminished by interrupting the insulin signaling and by ouabain, consistent with a glucose-induced hyperpolarization of V_m mediated through an autocrine inhibitory effect of insulin on Na/K ATPase activity.

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0064

Snapin, a collectrin binding protein, regulates insulin secretion

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Background and Aims: Mutations in the hepatocyte nuclear factor-1α (HNF-1α) gene cause a form of maturity onset diabetes of the young (MODY3) that is characterized by impaired insulin secretion. We have recently reported that collectrin, a novel target of HNF-1α in pancreatic β-cells, controls insulin exocytosis by interacting with snapin. Although snapin has been suggested to be involved in neurotransmitter secretion, the precise role of snapin in insulin secretion remains unclear. The present study is aimed to investigate the effects of snapin in insulin secretion.

Materials and Methods: Expression of snapin was examined by Northern blotting and immunohistochemistry. Gene expression of snapin in mouse islets was studied by quantitative RT–PCR. To study the effect of snapin on insulin secretion, the expression of snapin in INS-1 cells was suppressed by siRNA and glucose stimulated insulin secretion was examined. Functional interaction betseen snapin and collectrin was explored by transiently cotransfecting INS-1 cells with snapin and/or collectrin expression vector together with human GH expression vector. GST pull-down assay was performed to study the interaction of snapin and SNARE proteins.

Results: Immunohistochemical analysis revealed that snapin was expressed by pancreatic β-cells, but was not detected in exocrine cells. Snapin gene expression in the islets was not changed by feeding. Insulin secreton stimulated by 22 mM glucose was significantly inhibited (73.1% of control, p<0.05) in INS-1 cells by suppression of snapin (63.0% of control). GH secretion assay revealed that while overexpression of snapin alone did not increase hGH secretion, co-transfection of collectrin and snapin significantly increased hGH secretion than transfection of collectrin alone (162.5±7.1% vs 139.3±11.1%, percent to control, p<0.05). GST pull-down study revealed that snapin bound to SNAP25 as well as collectrin.

Conclusion: These data suggest that snapin regulates insulin secretion by interacting with collectrin.

0065

Stimulation of exocytosis by the miR-375 target protein myotrophin in insulin-secreting cells

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Introduction and Aims: Micro-RNAs (miRNAs) are short stretches of non-coding RNA, which negatively regulates specific target mRNAs. We have earlier described (Poy et al 2004, Nature 432:226–230) the finding of a miRNA, miR-375, which is expressed in pancreatic β-cells. In that study, it was shown that the exocytotic response, measured using the patch clamp technique in combination with capacitance measurements, was markedly reduced in single β-cells overexpressing miR-375. This effect was specific to β-cells as no change in the exocytotic response from α-cells was detected. To elucidate the target mRNA for miR-375, bioinformatic studies was performed. They revealed a series of potential target genes, of which myotrophin (Mtpn) was the most interesting. Myotrophin is a cytoplasmic protein expressed at high levels in the pancreatic β-cells. The aim of this study is to investigate by which meachanisms myotrophin regulates insulin secretion.

Material and Methods: Silencing RNA (siRNA) aimed towards myotrophin (NM_008098) was designed by Ambion (UK) and cloned into pRNAT-H1.1/Shuttle (Genscript, USA). Exocytosis was monitored as increases in membrane capacitance (ΔC_m) using the standard whole-cell configuration of the patch-clamp technique. The cells were transfected with the appropriate construct 48 h prior to experiments. Individual insulin-containing LDCVs were visualized using TIRF (total internal reflection of fluorescence)-microscope.
Results: To investigate the effects of myotrophin on insulin secretion, siRNA directed against myotrophin (siMtpn) was introduced into single insulin-secreting cells. Exocytosis was evoked by a train of ten 500-ms depolarizations from −70 to 0 mV. In the siMtpn treated cells the exocytotic response averaged 93±21 fF (n=8) while in the control cells (treated with a scrambled non homologous sequence) the same response averaged 177±32 fF (n=12; P<0.05). Further analysis of the exocytotic response revealed that the average capacitance increase in the initial depolarization amounted to 25±5 fF and 59±13 fF (P<0.01) in the siMtpn treated cells and control cells, respectively. Similarly, the capacitance increase during the last nine depolarizations was reduced from 118±31 fF under control conditions to 68±19 fF in the presence of siMtpn. To investigate if Mtpn is involved in the docking and/or priming of the insulin-containing vesicles, the time-dependent reduction of docked vesicles upon glucose-stimulation was evaluated using TIRF-microscopy. The insulin vesicles were labelled with IAPP-emerald. The preliminary results indicate that the relative number of docked granules 5 minutes after glucose-stimulation (15 mM) was reduced 67±7% (n=4) in cells transfected with siMtpn. This is to be compared with the control situation where the corresponding reduction amounted to only 40±10% (n=3, P<0.05).
Conclusions: Exocytosis as well as number of docked vesicles during glucose stimulation is reduced in cells treated with siMtpn suggesting a decreased ability in the treated cells do dock and/or prime the vesicles. Accordingly, we hypothesise that myotrophin stimulates the docking/priming of insulin-containing LDCVs.
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0066

Expression of $GABA_A$ receptor Cl^- channels and glucose-dependent GABA release in human pancreatic islets

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Background and Aims: Human pancreatic β-cells contain high concentrations of the neurotransmitter γ-aminobutyric acid (GABA). Previous work on rodent islets has shown that GABA is secreted from β-cells by regulated exocytosis and may act as a paracrine and autocrine regulator of hormone secretion. We now studied the expression of GABA receptors and GABA release in human pancreatic islets.
Materials and Methods: Patch-clamp experiments were performed on dispersed human pancreatic islet cells. To detect expression of endogenous $GABA_A$ receptors, GABA (1 mM) was rapidly applied to the surface of single cells using an oocyte injection system. After patch-clamping, the cell type was determined by immunocytochemistry. For studying GABA secretion from single β-cells or cell clusters, (auto-) synapses were created by infecting cells with adenoviral vectors encoding the $GABA_A$ receptor α_1 and β_1 subunit, respectively. Exocytosis of GABA-containing vesicles was detected as transient inward currents sensitive to the $GABA_A$ receptor antagonists bicuculline (100 μM) or SR95531 (10 μM).
Results: Expression of $GABA_A$ receptor Cl^- channels was detected in β-, α- and δ-cells. The current density was higher in δ-cells (207±126 pA/pF, receptors detected in 4 out of 4 cells) than in β-cells (12±4 pA/pF, 9 out of 18 cells) and α-cells (3 pA/pF, n=1). The currents could be blocked by SR95531 (50 μM). Exocytotic release of GABA was observed in single β-cells infused with buffer containing 3 μM free Ca^{2+} (n=7 from 3 donors). With EGTA (50 μM) containing intracellular solution, GABA release was triggered by voltage-clamp depolarisations from −70 to 0 mV (n=3). GABA exocytosis was also studied in islet cell clusters. The patch-clamped cell was held at −70 mV and infused with solution containing 10 mM EGTA, thus serving as a "sniffer" cell for release from neighbouring cells. Vesicular release of GABA was rarely observed at 5 mM glucose in the extracellular medium (0.5±0.3 events/min) and was strongly stimulated by increasing the glucose concentration to 10 mM (39±14 events/min 2 min after wash-in, p<0.05, n=4). At 4 mM glucose, exocytosis of GABA was triggered by addition of the K_{ATP} channel antagonist tolbutamide (100 μM; n=2).
Conclusion: We show here that human β-cells secrete GABA by regulated exocytosis. We demonstrate, for the first time in primary β-cells, that GABA exocytosis is stimulated by glucose. Expression of functional $GABA_A$ receptor Cl^- channels was found in the 3 major islet cell types. These findings constitute the basis for a putative function of GABA as a paracrine and/or autocrine transmitter in human islets.
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OP 12

Animal models of type 2 diabetes mellitus and insulin resistance

0067

Cardiac overexpression of adipose differentiation-related protein (ADRP) leads to myocardial steatosis

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Background and Aims: Diabetic cardiomyopathy is characterized by intracellular accumulation of triglyceride (TG) droplets that leads to cellular apoptosis and eventually cardiac dysfunction. The adipose differentiation-related protein (ADRP) is a lipid droplet-associated molecule expressed in many tissues including heart, although its patho-physiological function remains uncertain. We have found that cardiac ADRP expression is upregulated in concert with cardiac TG accumulation upon fasting, while this physiological regulation is impaired in diabetes. To explore the function of cardiac ADRP, we have generated transgenic mice with heart-specific overexpression of ADRP.
Materials and Methods: The cDNA of mouse ADRP and enhanced green fluorescent protein was subcloned under a promoter of myosin heavy-chain-α. The founders were backcrossed with C56BL/6 for 6 to 7 generations and heterozygous transgenic (Tg) mice and their Wildtype (Wt) littermates were studied. The expression of ADRP was analyzed by Western blotting and cardiac lipid content was measured enzymatically. Histological studies were performed using light and electron microscopy. To study cardiac uptake of fatty acids (FA), glucose and very low-density lipoprotein (VLDL), ^{123}I-beta-methyl iodophenyl pentadecanoic acid (BMIPP), ^{14}C-deoxy-D-glucose (DG) and ^{125}I-rabbit VLDL were injected into animals. Cardiac gene expressions were analyzed by quantitative RT–PCR (qRT–PCR).
Results: We have established 2 Tg lines, AD17 and AD19, that showed a high level of heart-specific ADRP expression on Western blotting. Light microscopy with oil red-O staining showed that Tg mice had many visible lipid droplets in cardiomyocytes. Electron microscopy revealed these lipid droplets as bubbly chains around clusters of mitochondria. Cardiac TG and FA contents were 8 times and 40% higher, respectively, in fed Tg mice compared to Wt mice. Interestingly, 24 h-fasted Tg mice showed 80% lower ADRP mRNA expression and 40% lower TG content compared with fed Tg mice, whereas Wt mice had higher ADRP expression and TG content with fasting than feeding. Cardiac ^{14}C-DG uptake was markedly reduced with fasting compared to fed condition in both genotypes, while no significant difference were seen between Tg and Wt. Cardiac uptake of ^{123}I-BMIPP and ^{125}I-VLDL were also similar in Tg compared with Wt mice in any conditions. qRT-PCR showed that expression of carnitine-palmitoyle transferase-1 (CPT-1) and uncoupling protein-2 (UCP-2) were significantly upregulated in Wt mice upon fasting, but this induction was blunted in Tg mice.
Conclusion: Cardiac overexpression of ADRP is sufficient to induce intracellular accumulation of TG droplets that histologically mimics diabetic cardiomyopathy. Although cardiac uptake of glucose, FA and VLDL were not altered, the blunted induction of CPT-1 and UCP-2 in Tg mice suggets a potential function of ADRP in controlling FA metabolism in the heart. Thus, cardiac ADRP could be a target to modulate diabetic cardiomyopathy.
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0068

***In vivo* lipoprotein lipase-mediated triglyceride metabolism in the human heart**

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Background and Aims: Animal studies have shown that circulating triglycerides are a major source of energy for the myocardium, and that excess triglyceride availability may lead to myocardial steatosis and cardiac dysfunction. Although the human heart is known to contain lipoprotein lipase (LPL), virtually no information is available regarding the possible uptake of circulating triglycerides by this tissue in humans.
Materials and Methods: We studied postabsorptive non-diabetic adults (N=6) during diagnostic coronary angiography. [1-^{14}C] oleate and a lipid emulsion labeled with [^{3}H] triolein were infused in tracer amounts to steady-state concentrations. Paired arterial and coronary sinus samples were taken every 4 min for 20 min. Systemic and myocardial kinetics, including the production of [^{3}H] oleate generated by the action of LPL on the infused triglyceride tracer, were determined. Fractional spillover of LPL-generated fatty acids was calculated as the rate of appearance (R_a) of [^{3}H] oleate ÷ the disappearance rate of the [^{3}H] triglyceride tracer, both for whole body and for myocardium.

Results: The coronary simus hematocrit was higher than arterial (36.5±1.3% vs 35.9±1.4%, P<0.03). Because of this, small corrections for hemoconcentration were made for plasma FFA and triglyceride concentrations in individual subjects. Plasma total free fatty acid concentrations were lower in the coronary sinus than in the artery (507±119 vs 653±106 μmol/L, P<0.001). [^{14}C] oleate specific activity was lower in the coronary sinus than in the artery (1.20±0.17 vs 1.34±0.18 dpm/nmol, P<0.05), whereas there was no difference in [^{3}H] oleate specific activity (4.85±0.97 vs 4.61±0.96 dpm/nmol, P=NS). Systemic oleate R_a was 2.7±0.6 μmol•kg^{-1}•min^{-1}. Myocardial production of oleate was 0.5±0.1% of systemic R_a. The fractional myocardial extraction of radiolabeled oleate was higher than that of radiolabeled triglyceride (34±5% vs 11±3%, P<0.001). The contribution of the heart to whole body disappearance of free fatty acids and triglycerides was 3.1±1.1% and 2.1±0.7%, respectively. Systemic fractional spillover of LPL-generated fatty acids was higher than myocardial (49±7% vs 20±3%, P<0.02).

Conclusion: These results indicate that in humans, myocardial LPL acts on circulating lipoproteins, resulting in uptake of fatty acids from triglycerides. Systemically, the heart is a minor site of uptake for both free fatty acids and triglycerides. It is also a minor source of systemic FFA R_a. Myocardial uptake of LPL-generated triglyceride fatty acids is relatively efficient compared with whole body uptake. Additional study is needed to determine whether myocardial triglyceride uptake is increased in individuals with hypertriglyceridemia, and whether it increases after ingestion of meals containing fat.

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0069

Skeletal muscle mitochondrial function and intramyocellular lipids in the diabetic Zucker rat: baseline data of a longitudinal study using *in vivo* ^{31}P and ^{1}H MR spectroscopy

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Background and Aims: Various mechanisms have been proposed to explain the relation between mitochondrial dysfunction and insulin resistance, often involving a role for intramyocellular lipids (IMCL). Data supporting these different hypotheses are derived from cross-sectional studies, making it difficult to determine whether mitochondrial dysfunction is either a cause or a consequence of insulin resistance. Thus, we investigate both mitochondrial function and IMCL levels using a longitudinal study design in the growing diabetic ZDF rat, a rodent model with progressive loss of insulin sensitivity during maturation resulting in overt diabetes in adulthood.

Materials and Methods: ZDF (fa/fa) (n=10) and control (fa/+) (n=11) rats are observed during the development of type 2 diabetes. At distinct time points during the pathogenesis (pre-diabetic: 6 weeks, diabetic: 12 weeks and long-term diabetic: 18 weeks of age) mitochondrial function and IMCL are measured using non-invasive ^{31}P MR spectroscopy (MRS) and ^{1}H MRS, respectively. Mitochondrial function was measured in tibialis anterior muscle (TA) as post-exercise phosphocreatine (PCr) recovery time constant (τ_{PCr}) using ^{31}P MRS after in vivo electrical stimulation. ^{1}H MRS is used to measure IMCL levels in the white (ventral) and red (dorsal) TA.

Results: Baseline data at 6 weeks of age are shown in table 1. Fasting plasma glucose levels and in vivo mitochondrial oxidative capacity (τ_{PCr}) was similar in fa/fa and fa/+rats. The fa/fa rats had a higher body mass and higher food intake compared to the fa/+animals (all p<0.001). The in vivo ^{1}H MRS measurements clearly showed higher IMCL levels in both the white and red TA (all p<0.001 for fa/fa vs. fa/+).

Conclusion: At 6 weeks of age, the fa/fa rats are hyperphagic, have a higher body weight and also IMCL content was already higher in fa/fa rats. Plasma glucose levels in the fa/fa rats were still normal. Interestingly, despite obviously higher IMCL levels, the fa/fa rats did not show an impaired in vivo skeletal muscle mitochondrial function since τ_{PCr} was the same as in fa/+animals. Overall, these results suggest that in the pre-diabetic stage, fa/fa animals do not have an impaired skeletal muscle mitochondrial function. Mitochondrial function and IMCL content will be monitored during the pathogenesis of type 2 diabetes and data at 12 and 18 weeks of age will be shown.

	Body weight (gr)	Food intake (gr)	Fasting glucose (mM)	IMCL white TA (% of H_2O signal)	IMCL red TA (% of H2O signal)	τp_{Cr} (s)
fa/fa (n=10)	193.6±1.9*	22.7±1.0*	5.3±0.3	0.46± 0.03*	0.80± 0.04*	45.0±4.4
fa/+ (n=11)	147.2±3.3	13.8±0.2	4.7±0.4	0.16± 0.02	0.34± 0.01	45.6±3.0

Typical parameters at 6 weeks of age. IMCL=intramyocellular lipids, TA=tibialis anterior muscle, τp_{Cr}=phosphocreatine recovery time constant, *=p>0.001 vs. Fa/+

0070

Tg972 mice show a global impairment of glucose homeostasis and insulin action

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Background and Aims: Several association studies have suggested that the G972 variant of the IRS-1 gene increase the risk for diabetes, obesity and atherosclerosis. In the last decade, we have addressed the functional significance of G972R variant in *in vitro* models, showing that the presence of this variant results in an impaired association of IRS-1 with the p85 subunit of PI3K , impaired glucose metabolism at the level of skeletal muscle and impaired insulin secretion and survival in pancreatic beta-cells. More recently we have genearated a transgenic mouse model overexpressing G972R IRS-1 under the control of the CMV promoter (Tg972) to address the impact of this variant on insulin resistance pathogenesis in vivo. Preliminary data showed that Tg972 mice develop age-related hyperglycemia and impaired glucose tolerance. On the basis of these preliminary findings, we proceeded to investigate the contribution of different insulin-sensitive tissues to the worsening of glucose metabolism in these mice.
Materials and Methods: We performed glucose tolerance tests incorporating the tracer ^{3}H-2-deoxyglucose to evaluate tissue specific glucose utilization and western blot assays to assess insulin signaling pathway activation in the different tissues. To specifically assess insulin secretion in these mice we purified pancreatic islets from Tg972 mice and their WT littermates and evaluated insulin content and insulin release after glucose stimulation from the isolated islets.
Results: We demonstrated that glucose uptake was markedly impaired in both muscle and adipose tissue from Tg972 compared to WT littermates ($p<0.05$) and glycogen incorporation was significantly reduced in liver from Tg972 ($p<0.01$). These changes were paralleled by a reduced activation of the insulin signaling pathway; in fact, despite an increased IRS-1 expression and total pTyr phosphorylation in all the three tissues, we observed a decreased IRS-1 associated PI3K activity and Akt phosphorylation ($p<0.001$). Furthermore it has recently been demonstrated that the introduction of the G972 variant in a recombinant peptide comprising amino acids 925–1008 from IRS-1 caused a reduction of Tyr phosphorylation on two specific residues Tyr941 and Tyr989, which are known binding sites for the p85alpha regulatory subunit. Using tissue lysates from Tg972 mice we were able to demonstrated for the first time in vivo a 70% reduction of Tyr ^{989} phosphorylation ($p<0.001$). To examine whether the mutation under study affects insulin secretion, we purified islets from Tg972 mice and WT littermates, and incubated them in the presence of increasing glucose concentration. Glucose-induced insulin secretion was significantly decreased in Tg972 islets at all the concentrations tested ($p<0.01$).
Conclusion: Tg972 show a global impairment of insulin signaling and action in all the key target tissues, thus reproducing the alterations observed in insulin resistance and glucose intolerance states and therefore representing an excellent model to study these conditions.
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0071

Adenosine A_{2B} receptor knock-out mice display an improved metabolic phenotype

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Background and Aims: The adenosine A_{2B} receptor is a G-protein coupled receptor expressed in liver, muscle, and other tissues. Several reports suggest that A_{2B} receptor antagonists could be used for diabetes treatment by supressing hepatic glucose output and increasing insulin-mediated glucose uptake in skeletal muscle, resulting in improved glycemic control. To further evaluate this concept, and to determine other consequences of A_{2B} receptor antagonism, we characterized A_{2B} knock-out mice.
Materials and Methods: Adenosine A_{2B} knockout mice (50%129ola+50% C57BL/6N) were obtained from Deltagen and crossed with C57BL/6N mice to a hybrid background of 80–95%. Het x Het matings produced the homozygous $-/-$ (KO) male and female mice used for the study. Age and sex-matched littermates served as wild-type (WT) controls. Animals were studied at 11–28 weeks of age (n=4–25/group). The phenotype analysis consisted of>40 bioassays designed to evaluate effects on major physiologic systems (e.g. neurologic, cardiovascular, metabolic, endocrine, immune, inflammatory, behavioral, musculoskeletal, urologic, and gastrointestinal) and metabolic response to a 7 week high fat diet (HFD, males only).
Results: There were several significant ($p<0.05$) findings for the adenosine A_{2B} receptor KO mice relative to WT controls.

A) *Standard diet*: *Body Composition*—Female KO mice demonstrated a marked reduction in fat mass (60%, $p<0.05$), resulting in a 40% ($p<0.05$) reduction in % body fat and 25% ($p<0.05$) increase in % lean mass compared to WT. Female KO mice also demonstrated increased ($p<0.05$) relative mass of the tibialis, EDL, gastrocnemius, and soleus muscles. *Tissue Glycogen*—Muscle glycogen concentration tended to increase in female KO mice ($p=0.09$), while liver glycogen was markedly reduced (87%, $p<0.05$) compared to WT. *Energy Expenditure*—Male KO mice showed a 20–25% ($p<0.05$) increase in O_2 consumption, CO_2 consumption, heat production and respiratory exchange ratio compared to WT.

B) *High-Fat Diet*: *Body Composition*—Male KO mice responded to the HFD with significantly ($p<0.05$) lower percent total body fat, and increased lean mass, percent lean mass, and a trend towards a decrease in fat mass compared to WT. *Plasma Chemistries and Hormones*—No differences in serum insulin levels were observed between male KO and WT mice. The KO mice had slightly ($p=0.09$) decreased adiponectin levels compared to WT before, but not after, the HFD. *Glucose Tolerance (GTT)*—No difference was observed prior to the HFD, but there was a trend for reduced glucose excursion (22%, $p=0.052$) during the GTT in the HFD KO mice compared to WT.

C) *Other Parameters*: No differences between KO and WT mice were observed for all other tests performed, except for inconsistent changes in bone endpoints, Elevated Maze test, RBCs and Phospholipid B, and Sebum Production.

Conclusions: A_{2B} receptor KO mice demonstrated a positive metabolic phenotype through reduced adiposity and increased lean body mass. GTT results suggested improved glucose tolerance in mice fed a HFD. There were also positive changes in energy expenditure, muscle mass, and tissue glycogen levels suggesting anti-obesity/anabolic effects. Further, there were no major adverse effects of the KO phenotype. Overall, these results suggest that reduction or antagonism of the adenosine A_{2B} receptor could be useful for the treatment of several metabolic disorders, including obesity, frailty, and type 2 diabetes.

0072

Cardiac energy metabolism in hormone-sensitive lipase knockout mice

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Background and Aims: Hormone-sensitive lipase (HSL) regulates intracellular lipolysis in many tissues including adipose, pancreatic beta cells and heart. HSL–knockout (KO) mice have been created and impaired adipocyte lipolysis was reported. Since free fatty acids (FA) are the major energy source for heart, and energy balance between glucose and fatty acids dramatically changes depending on nutritional condition, we asked how disruption of HSL affects cardiac energy metabolism. To clarify the impact of HSL–KO in cardiac metabolism, KO mice were fasted and refed, and the cardiac phenotypes investigated.

Materials and Methods: HSL–KO mice in C56BL/6 background aged 24 to 32 wks were studied. Homozygous KO mice and wildtype (Wt) littermates were fasted for 24 h and then refed for 3–6 h prior to the experiments. Plasma lipids, glucose and insulin concentration, and cardiac lipid contents were measured. Cardiac lipase activities were measured using radioactive substrates. Histological studies were performed by electron microscopy. To study cardiac uptake of FA, glucose and very low-density lipoprotein (VLDL), ^{123}I-beta-methyl iodophenyl pentadecanoic acid (BMIPP), ^{14}C-deoxy-D-glucose and rabbit VLDL whose tyrosine was labeled with ^{125}I, were injected into animals. Cardiac gene expressions were analyzed by quantitative RT–PCR.

Results: KO mice showed 30% higher plasma glucose and 9-times higher plasma insulin levels compared to Wt mice, indicating insulin resistance. Plasma FA concentration in Wt mice was elevated 2-fold with fasting, while this increase was not observed in KO mice. BMIPP uptake showed that cardiac FA influx paralleled plasma FA level; thus, KO mice had lower FA influx during fasting. Accordingly, upregulation of a FA-responsive gene, mitochondrial HMG CoA-synthase, was markedly blunted in KO mice in fasted and refed conditions. Although cardiac glucose uptake was similar between KO and Wt mice, cardiac VLDL uptake was 2-fold higher in KO than Wt mice during fasting, accompanied by a 2-fold increased cardiac lipoprotein lipase (LPL) activity. This LPL activation was not diminished by heparin perfusion, suggesting that LPL is located inside or on the surface of cardiomyocytes. Neutral cholesteryl esterase activity in KO heart was undetectable, while LPL and non-LPL triglyceride (TG) lipase activities were similar to Wt heart in fed condition. However, non-LPL TG lipase activity in KO heart was 40% lower and TG droplets accumulated during refeeding. The residual TG lipase in KO heart might be adipose TG lipase (ATGL) since its mRNA was expressed in heart and was upregulated 3-fold by fasting and refeeding in both KO and Wt.

Conclusion: Systemic disruption of HSL impairs FA release from adipose tissue upon fasting, and diminishes FA flux into cardiomyocytes. As compensation, cardiac

LPL is activated to facilitate VLDL-uptake presumably via lipoprotein receptors. HSL–KO affects activity of cardiac non-LPL TG lipases, including ATGL, and leads to aberrant lipid accumulation in cardiomyocytes.

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OP 13

Progression and mortality in type 2 diabetes

0073

The age at diagnosis predicts change in HbA_{1c} over time in patients with newly diagnosed type 2 diabetes

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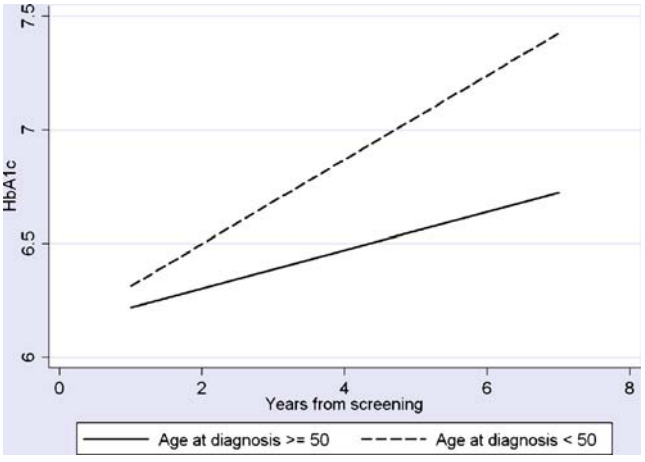
Background and Aims: Type 2 diabetes (T2D) is characterized by an initial decline followed by a progressive rise in blood glucose and HbA1c over time, which until now has been independent of mode of therapy. Although the underlying mechanisms are not fully understood, it has partially been ascribed to deterioration of β-cell function. The aim of this study was to identify non-genetic predictors of this deterioration with a future goal to also include genetic factors.

Materials and Methods: 1209 T2D patients from the local diabetes registry in southern Sweden were followed with repeated HbA1c measurements during 7 years after diagnosis. Fasting plasma glucose, fasting C-peptide and subsequently homeostasis model assessment of percent β-cell function (HOMA–B%) and HOMA of insulin resistence index (HOMA–IR) were measured at diagnosis in 585 of these patients. The relation between age at diagnosis, BMI at diagnosis, sex, HOMA–B% (estimated from C–peptide), HOMA–IR and ethnic background and changes in HbA1c during the follow up period adjusted for age, sex and BMI at diagnosis were studied using a generalized estimating equation procedure.

Results: HbA1c improved at one year of diagnosis from 7.6±2.18 to 6.3±1.4% (P=0.0001). Thereafter, HbA1c showed a progressive rise from 6.3±1.4 to 7.0±1.5% (P<0.000) during subsequent 6 years. The mean time to initiation of insulin therapy was 2.5 years. After 7 years 47% were on insulin therapy. In patients aged <50 years at diagnosis there was a steeper increase in HbA1c than in those with age at diagnosis ≥50 years (HR −0.10; CI −0.16–0.04, P=0.001). HOMA–B% was inversely associated with HbA1c values during the whole period (HR −0.004; CI −0.007–0.002, P<0.000). No relationship was observed between the rise in HbA1c over time and HOMA–IR, sex, BMI at diagnosis, or ethnicity (Scandinavian vs. non-Scandinavian origin).

Conclusion: We observed the expected rise in HbA1c over time in patients with newly diagnosed T2D. Early age at diagnosis (<50 years) was the strongest predictor of this secular increase in HbA1c concentrations and related to impaired β-cell function. This provides a basis to test whether genetic factors can modify these relationships.



0074

Early intensive insulin therapy on newly diagnosed type 2 diabetic patients: a long term follow-up study

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Background and Aims: In our previous study, we demonstrated that 2-week intensive insulin therapy with CSII could induce long-term glycemic control in newly diagnosed type 2 diabetic patients with severe hyperglycemia. Nearly one-half of the patients can maintain euglycemia on diet only for more than 12 months. 61 patients in the study had been followed for more than 24 months. We report here a study of these patients to analyze the follow-up data and assess the variables predictive of long-term near-normoglycemic remission.

Materials and Methods: 61 patients in our previous study (newly diagnosed type 2 diabetic patients with severe hyperglycemia who were treated with 2-week CSII and then on diet) had been followed for more than 24 months. Intravenous glucose tolerance tests (IVGTT) were performed. Insulin and C-peptide during IVGTT, FPG, glycosylated hemoglobin A_{1C} ($GHbA_{1C}$) and lipid profiles were measured. Group means were compared with Student's t test. A Kaplan–Meier survival analysis and

Cox hazard proportion analysis were used to determine the time to hyperglycemic relapse and variables predictive of long-term near-normoglycemic remission. The patients' initial data before and after CSII (including insulin and C-peptide during IVGTT, FPG, 2-h postbreakfast plasma glucose (PPG), $GHbA_{1c}$ and lipid profiles) were used for the Cox hazard proportion analysis.

Results: All of the patients (36 men and 25 women) with the initial FPG 14.3±4.8 mmol/L had been followed for more than 24 months. A total of 26 (18 men and 8 women) still maintained near-normoglycemic control without medication. 4 of these 26 patients had been in near-normoglycemic remission for more than 50 months. There were no differences in age, body mass index (BMI), $GHbA_{1C}$, FPG, PBG, insulin response, insulin resistance and lipid profiles before and after CSII between the patients still in remission (remission group) and those relapsed (relapse group). In this visit, the patients' final BMI were similar (remission vs relapse: 25.18±4.19 vs 24.50±3.76 kg/m^2). FPG of the patients in remission was significantly lower than that of the patients relapsed (5.88±0.88 vs 8.69±3.36 mmol/L), so as $GHbA_{1c}$ (6.07±0.70% vs 7.53±1.72%). And the acute insulin response of the remission group was markedly higher than that of the relapse group (1607.46±1221.85 vs 409.27±721.19 pmol/l/min). A Kaplan–Meier survival analysis gave a median time of 17 months (95%CI 11.67–22.33) to relapse. And the Cox models showed that the duration of near-normoglycemic remission was not significantly related to age, sex, FPG, BMI, insulin response, insulin resistance and weight change. The data suggested that the baseline LDL-cholesterol and PPG measured after CSII (on the following day at least 15 h after insulin cessation) were the risk factors affected the duration of near-nomoglycemic remission (odd ratio 2.471, 95% CI 1.282–4.760 and 1.410, 95% CI 1.136–1.752, respectively)

Conclusion: 26 of 61 newly diagnosed type 2 diabetic patients who were treated with 2-week CSII and then on diet had maintained near-normoglycemic remission without medication for more than 24 months. The median time to relapse was 17 months. The baseline LDL-cholesterol and PPG measured after CSII were the risk factors affected the duration of near-nomoglycemic remission.

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0075

Rosiglitazone combined with insulin therapy improves islet β cell function and increases serum GLP-1 level in type 2 diabetic patients

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Background and Aims: To investigate the effects of rosiglitazone(RSG) combined with insulin therapy on islet β cell function, especially on early phase of insulin secretion, and on serum glucagon-like peptide-1(GLP-1) level in type 2 diabetic patients (T2DP) who need to use insulin therapy to control blood glucose.

Materials and Methods: T2DP who need to use insulin therapy were selected. According to age, sex, diabetic duration, glycosylated hemoglobin A_{1C} (HbA_{1C}), matched-control study (1:1) was used. One was study group and the other was control group. The study group injected insulin and combined with RSG (4 mg/day) orally, the control group only used insulin. The therapy lasted for 3 months. 100 g carbohydrate (steamed bread) challenge of C-peptide (CP) releasing test was performed to evaluate β cell function. Other indices such as serum GLP-1(0 min, 30 min, 120 min), HbA_{1C}, tumor necrosis factor(TNF) -α, urinary ratio of albumin to creatinine (UA/Cr), free fatty acid(FFA), triglycerides (TG), high density lipoprotein cholesterol(HDL-ch) and low density lipoprotein cholesterol(LDL-ch) were detected.

Results: Thirty patients were included in the study and 28 patients finished follow-up. At the end, in the study group (n=14) serum levels of TG decreased[(1.98±1.20 vs 1.61±0.72)mmol/L, p=0.049], HDL-ch increased [(1.15±0.23 vs 1.36±0.28) mmol/L, p<0.001], FFA decreased [(663.14±88.18 vs 499.48±94.36) μmmol/L, p<0.001] significantly; In the control group (n=14) these indices were not changed significantly. The diastolic pressure in the study group decreased [(77.14±12.45 vs 74.50±5.92)mmHg,p=0.008], the blood pressure of control group did not have significant changes. Indices of early phase insulin secretion(EPIS) in the study group were improved significantly [CP30 min (0.63±0.23 vs 0.84±0.33) mmol/L, p=0.002, AUCcp30 (15.51±6.05 vs 19.85±7.19) mmol.min. L^{-1},p=0.000,ΔCP30/ΔG30(0.07±0.05 vs 0.14±0.10,p=0.02)]. Other indices of β cell function were also improved. Some indices of GLP-1 in the study group were increased significantly [GLP-130 min (24.45±1.23 vs 25.60±1.13)pmol/L, p=0.000, AUCglp30 (637.82±53.60 vs 659.68±50.25)pmol.min.L^{-1},p=0.000, AUCglp120(2601.43±184.41 vs 2686.29±171.07) pmol.min. L^{-1},p=0.000]. TNF -α[(7.92±1.60 vs 7.12±1.00) fmol/L, p=0.032] and ratio of UA/Cr [(42.59±27.43 vs 35.81±25.66) μg/mg, p=0.047] in the study group decreased significantly, and these indices in the control group were not changed significantly.

Conclusion: Rosiglitazone may improve islet β cell function including EPIS in T2DP who need insulin therapy, the protective effects of RSG on β cell might relate to the

increase of GLP-1 secretion partly except for the decrease of IR.

Supported by the Ethics Committee of West China Hospital

0076

Triple oral therapy with pioglitazone decreases the requirement for insulin therapy: a 42-week, double-blind, randomised, placebo-controlled study

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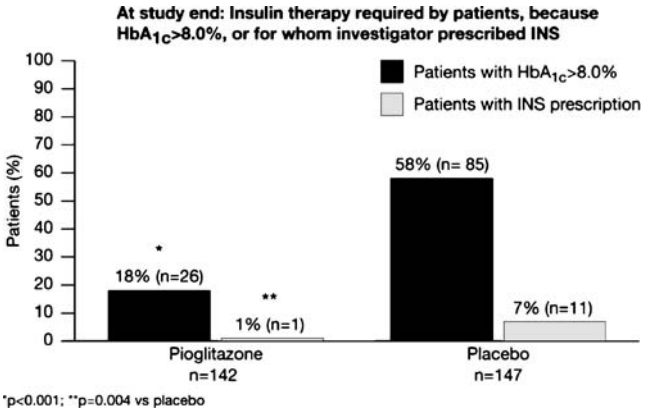
Background and Aims: According to current guidelines in France, insulin should be started in patients taking dual oral antidiabetic therapy who fail to achieve HbA_{1c} <8%. In the PROactive study, pioglitazone (PIO), a thiazolidinedione, has been shown to delay the need for permanent insulin use. There is, however, at present no clear evidence that adding PIO to failing metformin (MET) and sulphonylurea (SU) dual therapy postpones the need for exogenous insulin (INS). We evaluated the effect on the requirement for INS therapy of adding PIO to MET/SU dual therapy in inadequately controlled INS-naïve patients with Type 2 diabetes.

Materials and Methods: This prospective, double-blind, placebo (PBO)-controlled study, performed in 57 French centres, randomised 299 patients with Type 2 diabetes previously treated with MET (≥1700 mg/day) and SU (maximal dose) for more than 3 months and HbA_{1c} between 7.0% and 9.5%. After a 4-week run-in, patients received either PIO 30 mg once daily or PBO. After 3 months, if HbA_{1c}>6.5%, PIO or PBO was up-titrated to 45 mg for the remaining 4 months. Endpoints included the proportion of patients 1) requiring INS therapy at study end (HbA_{1c}>8%) and 2) for whom the investigator prescribed INS at study end.

Results: Overall, 289 patients (mean age 60±9 years, mean duration Type 2 diabetes 12±8 years) had at least 3 months' study treatment. At study end, 92% of the PIO group received 45 mg once daily and 82% of the PIO group versus 42% of the PBO group had HbA_{1c}≤8%.

At study end, one patient in the PIO group was prescribed INS in addition to oral therapy versus 11 patients in PBO group: nine in addition to oral therapy and two transferred to INS alone. After the study, the majority of investigators preferred to continue triple oral therapy than to prescribe INS. Triple oral therapy was prescribed to 67.5% of the PIO patients versus 58.5% of PBO group, dual therapy 30.0% versus 38.8% and monotherapy 0.7% versus 1.4%.



Conclusion: The addition of PIO to MET and SU significantly reduced the requirement for INS, with significantly more patients on PIO than PBO achieving HbA_{1c}≤8.0% after 7 months' treatment. Of interest was the disparity between clinical practice and clinical guidelines for the introduction of INS therapy in patients requiring tighter glycaemic control.

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0077

Lifestyle changes according to general guidelines on inflammation in subjects with impaired glucose tolerance: the SLIM project

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Background and Aims: To evaluate the effect of changes in C-reactive protein (CRP) and Interleukin 6 (IL-6) on changes in 2 h glucose levels (after an OGTT) and insulin sensitivity (as assessed by HOMA–IR) in subjects with impaired glucose tolerance (IGT), who participate in a lifestyle intervention program.

Materials and Methods: We studied 104 IGT subjects, intervention group; n=51 (INT) and control group; n=53 (CON) at baseline and after 1 year of lifestyle intervention. Subjects underwent measurements of body composition, body fat distribution, glucose tolerance (OGTT), and maximal aerobic capacity (VO2 max). The intervention program included dietary advice and a physical activity program, based on general public health recommendations.

Results: One year lifestyle intervention improved VO2 max and insulin sensitivity and reduced saturated fat intake (−2.34±3.00 En% in INT versus −0.52±3.34 En% in CON, p=0.004), reduced body weight (−2.67±3.57 kg in INT versus −0.53±3.86 kg in CON, p=0.004) and improved glucose tolerance (−0.74±1.79 mM in INT versus +0.36±2.17 mM in CON, p=0.006). No differences were observed in the change of CRP and IL-6 between INT and CON, p=0.545 and p=0.559, respectively. Also, no associations were seen between changes in CRP and IL-6 and changes in HOMA–IR. However, one-year changes in CRP (−0.07±6.36 mg/l) and IL-6 (−0.30±2.05 μg/l) were positively associated with change in 2 h glucose levels (−0.18±2.06 mM), adjusted R square 7.8%, p=0.007 and adjusted R square 8.4%, p=0.005, even after correction for changes in saturated fat intake, body weight and VO2 max change.

Conclusion: Lifestyle intervention improves glucose tolerance and insulin sensitivity in impaired glucose tolerant subjects, indicating that it may be effective in the prevention of type 2 diabetes mellitus. Changes in CRP and IL-6 were positively associated with changes in 2 h glucose levels, independent of lifestyle factors like change in saturated fat intake, weight loss and change in VO2 max. Therefore, changes in CRP and IL-6 seem important independent mediators of changes in glucose tolerance and may be considered as 'nontraditional' risk factors for the development of the impaired glucose tolerant state and type 2 diabetes mellitus.

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0078

Three-year mortality in diabetic patients treated with different combinations if insulin secretagogues and metformin

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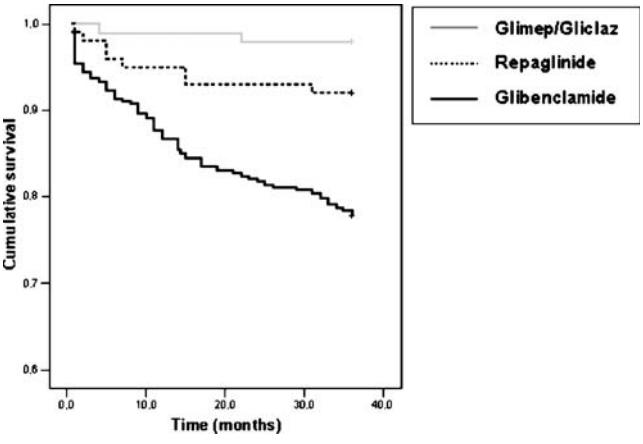
Background and Aims: Several studies have shown an increase of mortality in diabetic patients treated with combinations of sulphonylureas and biguanides. Comparisons between different insulin secretagogues in combination with metformin with respect to all-cause mortality have not been reported so far.

Materials and Methods: An observational cohort study was performed on a consecutive series of 2002 outpatients with type 2 diabetes mellitus. Of these patients, 696 (34.8%) were receiving combinations of insulin secretagogues and biguanides at enrolment. Three-year mortality was assessed through search in the City of Florence Registry Office.

Results: During follow-up, 295 deaths were recorded. Among patients on combined secretagogue and biguanide treatment, glibenclamide was associated with a significantly higher yearly mortality (8.7%) than repaglinide (3.1%; p=0.002), gliclazide (2.1%; p=0.001) and glimepiride (0.4%; p<0.0001). After adjusting for potential confounders (including age, duration of diabetes, BMI, lipid profile, HbA1c, insulin treatment, metformin doses, Charlson comorbidity score), mortality remained significantly higher in patients treated with combinations of glibenclamide and metformin when compared to those treated with different insulin secretagogues (OR with 95% CI: 2.09 [1.07;4.11]).

Conclusion: In the present study, sulphonylureas with greater selectivity for beta cell receptors, such as glimepiride and gliclazide were associated with a lower mortality, when used in combination with metformin, in comparison with glibenclamide. Safety of such combinations deserves further investigation.



OP 14

Central control of metabolism

0079

Mice lacking inhibitory leptin receptor signals are lean with normal endocrine function

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Background and Aims: The adipocyte-derived hormone, leptin, activates the long form leptin receptor (LRb) in regions of the brain to signal the status of body energy stores and regulate food intake, energy expenditure and neuroendocrine function. Lack of leptin or LRb results in obesity, infertility and growth retardation; the failure of elevated leptin levels to promote weight loss in common forms of obesity suggests that leptin-induced leptin resistance may promote obesity. LRb mediates intracellular signals via four pathways: (1) Tyr_{1138}-mediated activation of STAT3, (2) Tyr_{985}-mediated association with SHP-2 and SOCS3, (3) Tyr_{1077}-mediated phosphorylation of STAT5 and (4) via numerous tyrosine phosphorylation sites on the associated Jak2 molecule. We have previously shown that Tyr_{1138}/STAT3 is required for leptin regulation of energy homeostasis, but not growth or reproduction in mice. We have now examined the role of Tyr_{985} in LRb action *in vivo*.

Material and Methods: We have generated a line of genetically engineered mice in which the gene for LRb (*lepr*) is replaced by a mutant allele ($lepr^{l985}$) encoding a site-directed substitution mutation of Tyr_{985}. We investigated parameters such as body weight, food intake, leptin sensitivity, fertility and growth in these animals.

Results: Compared to wild-type mice, animals homozygous (*l/l*) for $lepr^{l985}$ display decreased weight and adiposity, with commensurately decreased levels of circulating leptin. In spite of these decreased leptin levels, l/l mice displayed increased hypothalamic LRb/STAT3 signaling and consumed less food than wild-type animals when fed a high fat diet. Furthermore, the *l/l* mice show increased sensitivity to exogenous leptin, as they demonstrated a greater reduction of body weight and food intake in response to leptin injections than did control animals. No impairments in growth or reproductive function were observed in l/l animals.

Conclusion: LRb signals to growth and reproduction are not mediated by Tyr_{985} or Tyr_{1138}, suggesting a role for LRb Tyr_{1077} or Jak2 mediated signals in these actions of leptin. The finding that Tyr_{985}-mediated signals decrease feeding and fat mass while not altering the growth and reproductive axes in mice suggests that leptin activates physiologically important autoinhibitory signals via LRb Tyr_{985}. These autoinhibitory signals act to attenuate leptin action and thus promote increased feeding and adiposity *in vivo* and are hence potential mediators of leptin resistance in obesity.

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0080

Central leptin infusion impairs insulin-stimulated GLUT4 translocation to plasma membrane in white adipose tissue in rats

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Background and Aims: Leptin modulates glucose homeostasis by acting as an insulin-sensitizing factor in most insulin target tissues. Nevertheless, insulin-dependent glucose uptake in white adipose tissue decreases after *in vivo* treatment with leptin. In the present work, we have analyzed the effects of central leptin on insulin-stimulated GLUT4 translocation in rat WAT.

Materials and Methods: 3-months-old Wistar rats were used throughout the study. Central leptin infusion was performed using osmotic pumps (Alzet) implanted in the right lateral cerebral ventricle, with a releasing rate of 1 μl/h with rat leptin (0.2 μg/day) or saline. During this time, rats were allowed free access to food and water. After 7 days the rats were subjected, under anesthesia, to an intravenous injection of insulin (10 U/kg body weight) or saline for 30 min and killed by decapitation. The epididymal WAT was rapidly dissected out, flash frozen in liquid nitrogen and stored at −70°C until use. Total RNA was isolated from EWAT and the mRNA expression of IR isoforms, IRS-1, and GLUT4 was studied by semiquantitative RT–PCR. IRS-1, Akt and GLUT4 protein content, and PY-IRS-1 and PS-Akt in different subcellular fractions of EWAT was analyzed by Western blotting.

Results: Central leptin infusion during 7 days reduce the mRNA expression of IR-B, IRS-1 and GLUT4 in the EWAT, without affecting the IR-A isoform. In addition, this treatment increased the basal phosphorylation levels on tyrosine and serine of both cytosolic IRS-1 and Akt, respectively. Nevertheless, central leptin infusion impaired the insulin-stimulated IRS-1 tyrosine phosphorylation and translocation to the cytosol, as well as Akt activation and GLUT4 translocation to the plasma membrane.

Conclusion: The observed effects of central leptin on the insulin signaling pathway in WAT may contribute to explain the reported decrease in insulin-stimulated glucose uptake in this tissue in leptin treated rats.

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0081

Central insulin response in Wistar rats: effects of ageing and food restriction

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Background and Aims: There is now compelling evidence supporting the role of insulin as a feed-back or adiposity signal, acting at central level. In the present report, we have studied the effects of ageing on central insulin action in Wistar rats. In spite of normal fasting serum levels of both, insulin and glucose, these animals present peripheral insulin resistance and central leptin resistance. Thus, we have explored whether or not these animals may also present alterations of the central insulin response. The fact that central leptin resistance associated with ageing in these rats can be ameliorated by food restriction also led us to investigate the effects of food restriction on central insulin action in these animals.

Materials and Methods: 3 and 24 months Wistar albino male rats were used in these experiments. The effects of central insulin administration on body weight and food intake was performed using osmotic pumps (Alzet) connected to the lateral ventricle, with a releasing rate of 1 μl/h with human-insulin (10 and 100 mU/day) or saline during 7 days. During this time, rats were allowed free access to food and water and body weight and food intake was measured. Central insulin signal transduction was studied after a single dose of insulin (10 mU) or vehicle into the lateral ventricle. 15 min after the treatment, rats were sacrificed and the hypothalamus was dissected, homogenated and used to check IR and GSK3 phosphorilation by imnmunoprecipitation and western blot analysis. The expression of IR also was studied by quantitative RTPCR and western blot in rat hypothalamus of young, old and old food restricted rats.

Food restriction. 20 to 21 months-old rats were placed in individual cages and were fed daily with an amount of chow equivalent to 75 to 80% of the normal food intake until they reached a reduction of about 25% of their body weight at the beginning of the experiment. Rats under this food restriction protocol during 3 month reached adiposity index similar to those of young rats.

Results: Insulin infusion during 7 days brought about a decrease in both body weight and food intake in a dose dependent manner in both young and old rats. Nevertheless, this effect was less evident in old rats and significance was only reached in young rats. IR and GSK3 phosphorylation was also decreased in old rats (43.7 and 28.78%, respectively, $P<0.001$) compared to young ones. No significant differences were appreciated in the expression of IR in rat hypothalamus with ageing. Food restriction increased central insulin responsiveness (body weight and food intake) in old rats as well as signal transduction.

Conclusion: The data presented herein demonstrate that aged rats present a lower response to central insulin. This can be explained by the impairment of the insulin signal transduction pathway. These alterations may contribute to the state of insulin resistance that takes place during ageing. The fact that food restriction improves central insulin responsiveness points to the role that adiposity may play in the development of insulin resistance associated with ageing.

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0082

Hypothalamic NPY during elderly and chronic food restriction: effect of central insulin

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Background and Aims: Male Wistar rats develop central leptin resistance during ageing which is ameliorated by chronic food restriction. Arcuate NPY serves as one of the downstream mediators of leptin's actions and arcuate NPY neurons are also a target for insulin action. In the present study we have analized the expression of NPY in the arcuate nucleus in a model of chronic food restriction in aging and the effect of central infusion of insulin. As NPY actions in the hypothalamus are both pre and postsynaptic, we also analized the expression on Y5 and Y2 NPY receptors.

Materials and Methods: 3 and 24 months Wistar albino male rats were used in these experiments. The effects of central insulin administration was performed using osmotic pumps (Alzet) connected to the lateral ventricle, with a releasing rate of 1 μl/h with human-insulin (10 and 100 mU/day) or saline during 7 days. During this time, rats were allowed free access to food and water. The expression of

NPY, Y_5 and Y_2 NPY receptors was studied by quantitative RT-PCR of rat hypothalamus of young, old and old food restricted rats. NPY expression in arcuate nucleus was also analized by immunohistochemistry and in situ hybridization using digoxigenin labeled riboprobes.
Food restriction. 20 to 21 months-old rats were placed in individual cages and were fed daily with an amount of chow equivalent to 75 to 80% of the normal food intake until they reached a reduction of about 25% of their body weight at the beginning of the experiment. Rats under this food restriction protocol during 3 month reached adiposity index similar to those of young rats.
Results: Aging is associated with a decrease in hypothalamic NPY expression analized by RT-PCR and correlated with less number of arcuate NPY cells analized by in situ hybridization histochemistry and also less inmunostaining for NPY. Chronic caloric restriction increases the expression of NPY in hypothalamus by RT-PCR and also by in situ hybridization in arcuate nucleus. Central infusion of insulin during 7 days reduce the expression of NPY in hypothalamus in a dose dependent manner in young but not in old rats. Caloric restriccion has no effect in the modulation of NPY by insulin in old rats. NPY Y_5 receptor expression decrease in old rats and food restriction increase its expression but at lower levels than in young animals. In young animals insulin infusion produce an upregulation of NPY Y_5 receptor and has the opposite effect in old annimals which is not changed by caloric restriction. NPY Y_2 receptor expression pressents the opossite changes than NPY Y_5 receptor during elderly and there is no changes during caloric restriction. Central infussion of insulin induce a decrease in the expression of NPY Y_2 in young and old rats without variation in food restricted animals.
Conclusion: Low levels of NPY arcuate expression in old animals cannot explain the same food intake in young than in old animals. The different response in young and old rats (also in food restricted annimals) in NPY expression after treatment with central insuline and the different regulation of pre and postsynaptic NPY receptors point to a central insuline resistance during aging.
Supported by Grants from Universidad Rey Juan Carlos and from Ministerio de Ciencia y Tecnología, Spain. We thank E. Oliveros and J. A. Mass for their technical assistance.

0083

Brain infusion of low dose glucosamine impairs glucose handling during ivGTT

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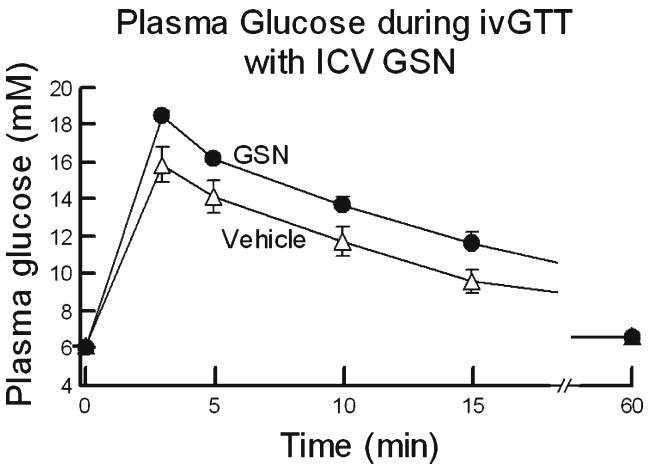
Background and Aims: Hypothalamic sensing of glucose/nutrition may be important in the integrated control of homeostatic metabolic processes such as appetite and hepatic glucose output. In order to sense changes in glucose, specialised neurones may use mechanisms used by pancreatic beta-cells such as the low affinity hexokinase, glucokinase (GK). We hypothesised that brain delivery of the GK inhibitor, glucosamine (GSN) would reduce brain sensing of glucose and reduce glucose handling during a glucose challenge.
Materials and Methods: We performed 3 sequential studies to look at the effects of brain delivery of GSN on glucose tolerance.
(1) We examined pooled Sprague Dawley rat hypothalamic preparations. At a glucose concentration of 3 mM, GK represented approximately 5% of total hypothalamic glucose phosphorylation and was selectively inhibited by 1 mM GSN (reduced to 52+7% activity compared with 100+1% for non-GK phosphorylation).
(2) We then studied overnight-fasted chronically catheterised (vein and brain ICV catheters) adult male rats. In pilot studies, a 30 minute ICV infusion of 75 nmol/min GSN resulted in an approximate 1 mM rise in hypothalamic hexosamine levels compared with vehicle (1.4+0.4 *vs* 0.2+0.1 mM, $p<0.001$) with no changes in levels in other brain areas or blood.
(3) Using this dose of GSN, we then examined the effects of 90 minute ICV infusion of GSN (n=7) or vehicle (n=7) on glucose and insulin responses to an intravenous glucose tolerance test (ivGTT- 0.5 g/kg) in chronically catheterised Sprague Dawley rats.
Results: Following ICV GSN, rats displayed impaired handling of glucose during the first minutes of ivGTT, with a significantly higher glucose peak (18.5+0.2 *vs* 15.8+0.9 mM, $p=0.02$) and $AUC_{0–10\ min}$(86+2 *vs* 66+7 mM.min, $p=0.02$). Despite these higher circulating glucose levels, insulin levels tended to be lower in ICV GSN rats (peak 3.6+0.5 *vs* 4.3+0.6 ug/l, p=NS).
Conclusion: Inhibition of hypothalamic GK reduced the efficiency of disposal of a glucose load supporting a role for brain GK-mediated glucose-sensing in the integrated responses to hyperglycaemia. The trend for a lower insulin response indicates that this may be mediated, at least in part, by a reduction in first phase insulin release suggesting a contribution from brain glucose-sensing in the integrated control of glucose-stimulated insulin release *in vivo*.



Supported by JDRF, Diabetes Research Wellness Foundation

0084

Central lipids overload increases NOS activity and modulate peripheral insulin secretion and action in response to glucose

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Background and Aims: We have previously shown that rats infused with lipids for 24 h towards brain displayed a hepatic insulin resistance and an increased GIIS (Glucose Induced Insulin Secretion), both due to changes in autonomic nervous activities. Some data showed that deleterious effects of fatty acids (lipotoxicity) implicate inflammatory Nitric Oxide (NO) pathway. So we aimed to test whether central NO could be involved in the early dysfunctions of glucose homeostasis induced by lipids.
Materials and Methods: Two groups of rats were infused during 24 hours into carotid artery towards brain, control group received saline with heparin (C), and the other group a lipids emulsion (Intralipid®) with heparin (IL). We measured hypothalamic Nitric Oxide Synthases (NOS) activity and mRNA expression for inducible NOS (iNOS, Ca^{2+} independent), and endothelial/neural NOS (eNOS, nNOS) in both group.
Then we treated rats with an acute injection of competitive NOS inhibitor *N_G-Monomethyl-L-Arginine Monoacetate* (L-NMMA) into carotid artery, just before the Intra-Venous Glucose Tolerance Test (IVGTT). We also injected L-NMMA during euglycemic-hyperinsulinemic clamps to test the effects of central NO production on glucose kinetics.
Results: The lipid infusion did not lead to any modifications of plasma triglycerides and free fatty acids. Food intake and body weight remained similar between groups. eNOS, nNOS and iNOS mRNA expression was unchanged in the hypothalamus of IL rats, whereas iNOS activity was significantly increased (+56%, $p<0,05$) , as well as eNOS and nNOS activities (+90%, $p<0,05$). L-NMMA treatment had no effect on glycaemia time courses during IVGTT and no effect on insulinaemia time course of C rats, whereas the increased GIIS characteristic of IL rats was normalized after L-NMMA injection. The clamps study showed a hepatic insulin resistance in IL rats, which is abolished following L-NMMA treatment.
Conclusion: These results suggest that NO pathway is involved in the central effect of lipid overload: the increase activity of NOS is probably responsible, at least in part, of the hepatic insulin resistance and the concomitant increase in insulin secretion in response to glucose. Indeed we shown that central NOS blockade restore normal GIIS and hepatic insulin sensitivity in IL group. We propose that induction of NO pathway could be at the crossroad of inflammatory process, lipotoxicity and autonomic control of glucose homeostasis and energy balance.

OP 15

Inflammation and arterial modelling

0085

Benfotiamine reduces the postprandial increase in serum inflammatory markers induced by dietary AGE (Advanced Glycation Endproducts) in patients with type 2 diabetes mellitus (T2DM)

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Background and Aims: In patients with T2DM, an AGE-rich diet can induce after 2–6 weeks persistent increases in inflammatory mediators (CRP, TNFα). Benfotiamine (BT), the liposoluble derivative of vitamin B1, has the capacity to inhibit AGE formation *in vitro* and *in vivo*. The acute effects of dietary AGE on inflammatory markers and their modulation by BT have not been studied until now.
Materials and Methods: Therefore, 19 T2DM inpatients (age: 55.4±2.2 years, HbA1c: 8.8±0.5%, without acute cardiovascular events within the previous 6 months)

received a standard diabetes diet for 9 days. On day 4 and 6, the acute effects of a high AGE or a low AGE meal (HAGE vs. LAGE, 15.100 vs. 2.750 kU AGE) on serum inflammatory markers (IL-6, IL-8) were studied in a randomized, cross-over, investigator-blinded design. In a subgroup of 11 patients the HAGE meal was repeated on day 9 after a 3-day pre-treatment with BT (Milgamma®, Wörwag, Germany): 3×350 mg/d on day 7 and 8, 1.050 mg on day 9, 1 h prior to the meal. Inflammatory markers (determined by ELISA, ImmuLite 2000, DPC Biermann, Bad Nauheim, Germany) were assessed after an overnight fast, prior and 2, 4 and 6 h after HAGE or LAGE. Both meals had the same ingredients (580 kcal, 54 g protein, 17 g lipids, 48 g carbohydrates) and differed only by the cooking conditions (temperature and time).

Results: IL-6 levels increased after HAGE by 100%, from 2.9±0.6 pg/ml at baseline to 3.9±1.1 (2 h), 3.4±0.6 (4 h) and 5.8±1.2*† (6 h) (*p<0.05 vs. baseline, †p<0.05 vs. LAGE), and changed transiently and non-significantly after LAGE, from 3.5±0.8 pg/ml at baseline to 2.9±0.4 (2 h), 3.1±0.4 (4 h) and 4.0±0.6 (6 h)(p=ns). BT pre-treatment could not prevent this increase, but reduced its extent to 69,5%: from 2.2±0.1 pg/ml at baseline to 2.2±0.1 (2 h), 2.3±0.2 (4 h) and 2.9±0.3* (6 h) (*p<0.05 vs. baseline).

IL-8 levels changed after HAGE from 9.5±0.9 pg/ml at baseline to 10.6±1.6 (2 h), 10.1±1.5 (4 h) and 10.4±1.6 (6 h) and after LAGE from 10.4±1.5 pg/ml at baseline to 8.9±0.9 (2 h), 8.0±0.7 (4 h) and 8.3±0.7 (6 h)(p=ns vs. baseline for all). Pre-treatment with benfotiamine before the repeated HAGE meal led to a persistent and significant decrease in IL-8 levels: from 9.7±0.8 pg/ml at baseline to 9.2±0.9 (2 h), 8.8±0.8* (4 h) and 8.6±0.7* (6 h)(p<0.05 vs. baseline).

Conclusion: Even a single real-life AGE-rich meal induces an acute increase in serum inflammatory markers (IL-6, IL-8) in T2DM, while a LAGE meal with identical ingredients does not. Pre-treatment with benfotiamine partly prevents the increase of IL-6 and induces a persistent decrease of IL-8. The benefits of long-term supplementation with benfotiamine remain to be established.

0086

Inflammation in epicardial adipose tissue: comparison of inflammatory mechanisms for production of adipocytokines in human epicardial and gluteo-femoral adipose tissue in CABG patients

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Background and Aims: Studies have established that adipose tissue distribution has significant impact on disease with central abdominal fat increasing both type 2 diabetes (T2DM) and cardiovascular disease (CVD) risk compared with gluteo-femoral fat. Whilst the relative pathogenic risk of abdominal and gluteo-femoral adipose tissue are apparent, few studies to date have investigated site specific nature of human epicardial adipose tissue compared with gluteo-femoral fat. Our recent studies have implicated epicardial adipose tissue (AT) as an important source of pro-inflammatory cytokines which may have important impact on myocardial function due to its proximity to the heart. Therefore the aim of this study using matched paired epicardial adipose tissue (AT) and gluteo-femoral (thigh) AT from patients undergoing coronary artery bypass grafting (CABG) patients was to examine (1) the key mediators of the innate immune pathway toll like receptors, TLR 2 and TLR 4 (2) the inflammatory signaling molecules nuclear factor kappa B and c-jun kinase (JNK) key mediators of adipocytokine production (3) and the mRNA expression of CD68 as a marker of macrophages investigating the role of macrophages in epicardial adipose tissue.

Materials and Methods: Epicardial and gluteo-femoral adipose tissue was obtained from subjects undergoing coronary artery bypass grafting (n=16, Age=66.7±6.7, BMI=28.4±3.6). Components of the NFκB pathway were analysed at a protein level by Western Blotting. Gene expression was analysed by quantitative RT-PCR using the ABI 7500 Real-Time PCR system.

Results: Gene expression data in the epicardial adipose tissue showed strong correlations between CD-68 with both TLR-2 and 4 (TLR-2 r=0.94, p<0.001; TLR-4 r=0.95, p<0.001) and also TNFα(r=0.81, p<0.001). Protein expression data of NFκB and the IKK complex showed epicardial AT had significantly higher NFκB, IKK-γ, IKK-β compared with thigh AT (NFκB: Epicardial 2.83±(SEM)0.37 vs Thigh: 1.69±0.37 p=0.06, IKKβ Epicardial 3.59±0.25 vs Thigh 1.50±0.33 p=0.01, IKKγ Epicardial 2.22±0.10 vs Thigh 1.02±0.03 p=0.001). In addition JNK protein expression data indicated a trend towards upregulation in epicardial AT compared with thigh (JNK: Epicardial 3.15±0.57 vs Thigh 1.35±0.21 p=NS, n=4).

Conclusion: In summary these studies highlight that epicardial adipose tissue has increased NFκB, and IKKβ an important determinant in inflammation activation compared with matched thigh tissue. We identified a close positive correlation between both TLR-2 and-4 affirms there known interaction in inflammation. Further our mRNA expression data of CD68 also implicates macrophage infiltration in this increased inflammatory state. As such increased levels of NFκB pathway observed in the epicardial depot may arise through activated macrophages in conjunction with the adipocytes, this is supported by the

strong correlations of mRNA expression of the NFκB target gene and TNF-α with CD-68. In conclusion, these studies highlight epicardial AT as a source of inflammatory cytokines mediated through NFκB and components of key inflammatory pathways and further highlights the role of the macrophage in inflammation within this tissue.
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0087

Plasma adiponectin levels are inversely associated with carotid intima media thickness and markers of insulin resistance in middle-aged men

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Background and Aims: Circulating plasma adiponectin is a peptide marker for insulin sensitivity and glucose metabolism, derived from fat cells. It is still largely unknown if adiponectin is also an independent marker for early atherosclerosis or not.
Materials and Methods: We measured plasma adiponectin levels in a sample of 384 middle-aged men and 524 middle-aged women, as a subgroup of the Malmö Diet Cancer–Cardiovascular Branch (MDC–CVB; n=6103) study. The subjects were sampled stratified for degree of insulin sensitivity as evaluated by the HOMA–IR index. Clinical examination (height, weight, waist and hip circumference, blood pressure (BP)) and fasting blood sampling (lipids, haemoglobin A_{1c} (HbA_{1c})) was carried out in all subjects. An ultrasound measurement of the right common carotid artery intima media thickness (IMT) was made by use of Accuson device. Plasma adiponectin was measured by a novel in-house (Aarhus) time-resolved immunofluorometric assay (TR-IFMA).
Results: When the distribution of adiponectin was stratified into sex-specific quartiles (Q1–Q4), men in Q4 differed from Q1 in higher mean age and HDL–cholesterol, but lower diastolic BP (DBP), HbA_{1c}, HOMA-index, BMI, and prevalence of diabetes. Women in adiponectin Q4 compared to Q1 had higher HDL-cholesterol, but lower HbA_{1c}, HOMA-index, BMI, waist-hip ratio and prevalence of diabetes. Mean IMT for men was significantly lower (p=0.03) in adiponectin Q4 as compared to Q1 when adjusted for age, waist circumference, smoking, HDL-cholesterol, and DBP in multiple regression analyses. When adding HbA_{1c} to the model, the association was no longer significant (p=0.08). For women no difference in IMT was noticed across adiponectin quartiles.
Conclusion: Plasma adiponectin is a marker of glucose metabolism and obesity and shows an inverse age-adjusted association with carotid ultrasound IMT in men, but not in women. This association is is attenuated after adjustment for metabolic risk factors.
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0088

Undiagnosed dysglycaemia and inflammation in cardiovascular disease

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Background and Aims: Cardiovascular (CV) disease is associated with increased levels of glucose and enhanced inflammation, but the prevalence of dysglycaemia in CV diseases is not fully known. We examined the prevalence of unknown dysglycaemia and its association with inflammation in Caucasian patients with ischemic vascular complications (coronary artery disease [CAD], cerebrovascular disease [CVD], peripheral artery disease [PAD]).
Materials and Methods: This case control study involved 149 patients (mean age 68 years) hospitalised for CAD (n=51), PAD (n=47) or CVD (n=51) and 59 control subjects (CTR, mean age 66 years) free from CV-disease. Anthropometric measures were similar among all groups. The prevalence of dysglycaemia according to WHO/ADA criteria (impaired fasting glycaemia, impaired glucose tolerance or diabetes mellitus) was assessed by a 75 g oral glucose tolerance test (OGTT). Inflammatory parameters were analysed in fasting serum samples.

Results: Dysglycaemia was found in 49%, 55% and 57% (Fig 1) among patients with CAD, CVD and PAD, respectively, all significantly higher than among controls (29%). The odds ratio for being dysglycaemic were 1.7 (95% CI: 1.04–2.77), 1.9 (1.19–3.06) and 2.0 (1.25–3.19) for CAD, CVD and PAD respectively. Inflammatory markers (the total leukocyte count, sTNF–R1, CRP) were elevated in patient groups (Table 1) while the opposite was true for the anti-inflammatory cytokine TGF-β1 and IGF-BP3 in patients with CAD. Inflammatory markers tended to parallel increasing blood glucose levels in all groups (not shown).
Conclusion: Undiagnosed dysglycaemia was common in patients with ischemic CV manifestations regardless of vascular bed involved. Inflammation was enhanced in patients with CV-complications and associated in a dosage-related-manner to glucose levels.

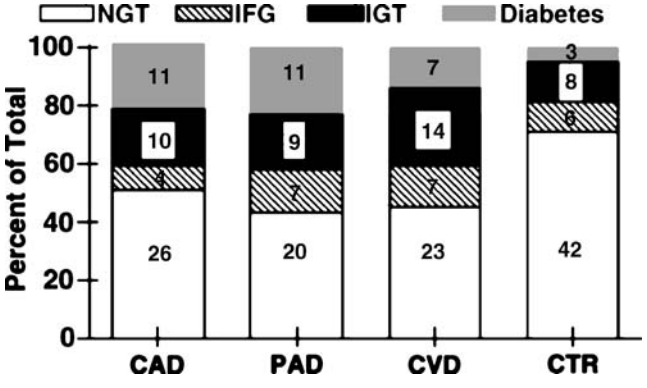

Table 1. Inflammatory markers of the different groups and controls.

	CAD	PAD	CVD	CTR
Total leukocyte count (10^9/l)	6.9±2.0**	7.2±1.8***	6.8±1.9**	5.9±1.4
CRP (mg/dl)	3.7	4.8*	3.7	2.8
(95%CI)	(2.2–5.2)	(3.3–6.4)	(2.3–5.1)	(1.8–3.7)
sTNF-R1 (pg/ml)	999±493*	1036±376**	965±539	850±283
TGF-B1 (ng/ml)	5.4±2.9**	6.5±3.4	6.6±3.8	7.4±3.6
IGF-1 (ng/ml)	62.9±24.3	78.7±28.6	72.4±26.0	70.5±29.5
IGFBP3 (μg/ml)	1.5±0.4*	1.5±0.4	1.6±0.4	1.6±0.4

Data are mean±SD unless otherwise stated. *p<0.05, **p<0.01 and *** p<0.001 versus CTR. CAD, coronary artery disease; PAD, peripheral artery disease; CVD, cerebrovascular disease; CTR, controls; CRP, C-reactive protein; sTNF-RI, soluble tumor necrosis factor receptor type I; TGF, transforming growth factor; IGF, insulin-like growth factor, IGFBP, IGF binding protein.

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0089

Additive anti-inflammatory effect of pioglitazone to simvastatin in non-diabetic cardiovascular disease, the role of insulin resistance

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Background and Aims: Low-grade inflammation (LGI) is a pathogenic factor for atherosclerosis. High sensitivity CRP (hs-CRP), matrix metalloproteinase 9 (MMP9), and plaminogen activator inhibitor (PAI-1) are markers of LGI, associated with cardiovascular disease (CVD). Statins and PPAR γ antagonists (i.e. pioglitazone) lower inflammatory markers and reduce CVD in type 2 diabetes. We aimed to test the safety and efficacy of the combination of pioglitazone and simvastatin versus each individual drug in non-diabetic subjects with CVD and high hs-CRP.
Materials and Methods: In a prospective, double blinded, double dummy, three arm parallel trial 125 subjects were randomly assigned to receive simvastatin or pioglitazone plus placebo or both drugs. Follow-up was 12 weeks. We tested the changes in hs-CRP by analysis of covariance (ANCOVA) with fixed effect factors for treatment group and baseline hs-CRP as covariate. The correlation between changes in hs-CRP and insulin resistance (HOMA) was calculated with Spearmans rank correlation test.
Results: At baseline there were no significant differences in the groups. Pioglitazone and simvastatin significantly reduced hs-CRP (3.64±2.42 to 2.48±1.77 mg/L and 3.26±2.02 to 2.81±2.11 mg/L); but the combined treatment had an additive effect (hs-CRP from 3.49±1.97 to 2.06±1.42 mg/L, p<0.0103). HOMA decreased in both groups on pioglitazone and the correlation between the changes in insulin sensitivity and in hs-CRP was significant (r=0.43; p<0.05). No serious adverse events occurred in any group. Additionally PAI-1 decreased significantly only in the pioglitazone groups and MMP 9 in the 3 groups
Conclusion: Pioglitazone, by reducing insulin resistance, has additive antiinflammatory effects to Simvastatin in non-diabetic subjects with CVD and high hs-CRP.
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0090

Divergent relationship between soluble TNF-alpha receptor 1 and 2, insulin resistance and endothelial function

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Background and Aims: Tumor necrosis factor-α (TNF-α) is a pro-inflammatory cytokine that is implicated in the pathogenesis of endothelium dysfunction and insulin resistance. Serum concentrations of soluble TNF-α receptor 1 and 2 (sTNFR1 and sTNFR2) are thought to reflect TNF-α action. We aimed to evaluate whether circulating sTNFR1 and 2 contribute to explain the complex effects of TNF-α on endothelial function independent of insulin resistance.

Materials and Methods: Circulating sTNFR1 and sTNFR2 concentrations were measured by EASIA. Insulin sensitivity (minimal model) and brachial vascular reactivity (high resolution external ultrasound) were assessed in 100 consecutive nondiabetic Caucasian men (70 with normal and 30 with impaired glucose tolerance).

Results: In subjects with normal glucose tolerance, serum sTNFR1 was an independent contributor to the variance of endothelium-dependent vasodilatation after adjusting for age, BMI, smoking status, systolic and diastolic blood pressure and insulin sensitivity ($p=0.002$). In fact, circulating sTNFR1 was positively associated with endothelium-dependent vasodilatation in these subjects ($r=0.291$, $p=0.02$). In contrast, serum sTNFR2 concentration was negatively associated with endothelium-dependent vasodilatation in all subjects as a whole (trend $r=-0.190$, $p=0.058$), and the latter association was especially significant in subjects with impaired glucose tolerance ($r=-0.366$, $p=0.047$). No significant associations were observed between endothelium-independent vasodilatation and serum sTNFR1 or sTNFR2 concentration.

Conclusion: TNF-α receptor shedding (circulating sTNFR1 and sTNFR2) could help to explain the complex effects of TNF-α on endothelial function.

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OP 16

Epidemiology of CVD in type 2 diabetes

0091

Use of sulphonylureas and mortality after myocardial infarction in diabetic patients: a Danish nationwide population-based study

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Background and Aims: Sulphonylureas (SUs) can block the ATP-sensitive potassium channels in the heart, thereby inhibiting the myoprotective effects of ischemic preconditioning (IP). However, in experimental studies, only glibenclamide inhibit IP, whereas newer SUs (gliclazide and glimepiride) do not abolish this protection. The clinical implications of these pharmacological differences are unclear as data from clinical studies are sparse, but in a preliminary regional Danish study, we found a lower risk of myocardial infarction (MI) with gliclazide and glimepiride when compared to other SUs and a trend towards a lower 30-day mortality among users of gliclazide compared to users of other SUs.

Material and Methods: We examined the mortality among users of "new" once-daily SUs (gliclazide and glimepiride) and "old" SUs (glibenclamide, glipizide and tolbutamide) in a nationwide population-based follow-up study in Denmark. We identified 54,835 patients with a first-time hospitalization for MI in Denmark in the period 1996–2002 using data from the Danish National Hospital Registry (DNHR). Among MI patients, data on diabetes and type of antidiabetic treatment were obtained from the DNHR and a national prescription database. Mortality was determined through the Civil Registration System. We estimated 30-day mortality rate ratios (MRRs) associated with the use of different SUs before MI adjusted for a range of prognostic factors including duration of diabetes, use of cardiovascular drugs and level of comorbidity, using Cox regression.

Results: Among MI patients, we identified a total of 6,644 patients with diabetes. Treatment with "new" SUs before

MI was associated with a decreased 30-day mortality both when compared to no pharmacotherapy for diabetes (see table below) and when compared to treatment with "old" SUs (adjusted MRR=0.75, 95% CI: 0.61–0.93, p=0.009). The apparent advantage in 30-day mortality of treatment with "new" SUs was also evident during the entire follow-up period (median follow-up time 1.68 years).

30-day MRRs according to antidiabetic treatment

Antidiabetic medication	Number	Deaths	Mortality	Adjusted MRR (95% CI)
No pharmacotherapy	1,636	375	22.9%	1.00 (reference)
"New" sulphonylureas	579	110	19.0%	0.81 (0.65–1.01)
-Glimepiride	465	90	19.4%	0.84 (0.66–1.06)
-Gliclazide	115	20	17.4%	0.75 (0.47–1.17)
"Old" sulphonylureas	1,526	395	25.9%	1.08 (0.93–1.27)
-Glibenclamide	776	193	24.9%	1.08 (0.90-1.30)
-Glipizide	378	108	28.6%	1.15 (0.92–1.43)
-Tolbutamide	387	101	26.1%	1.10 (0.87–1.38)

Conclusion: Our findings support the hypothesis that "new" SUs (gliclazide and glimepiride) have cardioprotective effects that may translate into improved survival after MI.

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0092

High risk of cardiovascular mortality in subjects with impaired fasting glucose is mostly explained by those who convert to diabetes

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Background and Aims: Impaired glucose regulation is considered a high-risk state for type 2 diabetes and cardiovascular disease (CVD). The American Diabetes Association (ADA) introduced diagnostic criteria for impaired fasting glucose (IFG) in 1997. In 2003, the ADA lowered the threshold for the definition of impaired fasting glucose (IFG) in order to increase the sensitivity of identifying subjects who will convert to diabetes within 10 years. However, the consequences of lowering this cut point of IFG for CVD risk were not considered. Our aim was to assess the impact of lowering the IFG cut point on the associated risk of CVD mortality and to evaluate whether this risk is explained by subjects who later convert to type 2 diabetes.

Materials and Methods: The risk of CVD mortality for subjects with impaired fasting glucose was studied in a population-based cohort, the Hoorn Study. A 75-g oral glucose tolerance test (OGTT) was administered at baseline (1989) and at the follow-up examination in 1996 (n=1428). Subjects were classified at baseline according to criteria of the ADA of 1997 (IFG if fasting plasma glucose (FPG) 6.1–7.0 mmol/l) and of 2003 (IFG if FPG 5.6–7.0 mmol/l). For the subjects who were classified as IFG at baseline, it was determined whether they had converted to diabetes or not by classifying them again in 1996. For CVD mortality in the period from 1996 until January 1st 2005, hazard ratios (HRs) and 95% confidence intervals (CIs) were obtained from Cox proportional hazards models (adjusted for age and sex).

Results: For the ADA-1997 criteria but not for the ADA-2003 criteria, IFG-subjects had a significantly higher CVD mortality risk than normal fasting glucose (NFG)-subjects (Table 1). For both sets of criteria, subjects who converted from IFG to diabetes had a more than two-fold risk at CVD mortality (ADA-2003: 2.14 (1.12–4.10) and ADA-1997: 2.47 (1.17–5.19), Table 1) than subjects who were classified as NFG. Subjects who remained IFG or had converted to NFG had only slightly (and not significantly) higher CVD mortality risks than subjects with NFG (ADA-2003: 1.15 (0.69–1.93) and ADA-1997: 1.50(0.72–3.15)).

Conclusion: The lower cut point for IFG (ADA-2003 criteria) results in a category of IFG which no longer represents a high-risk state of CVD. Furthermore, we showed that the association between IFG and the risk at CVD mortality is mostly explained by the conversion to diabetes.

Table 1. Age- and sex-adjusted hazard ratios of CVD mortality for glucose tolerance categories

	ADA-1997			ADA-2003		
	N	CVD mortality (n)	HR (95% CI)	N	CVD mortality (n)	HR (95% CI)
NFG	1217	58	1	878	39	1
IFG, all	149	16	1.87 (1.07–3.25)	488	35	1.37 (0.87–2.16)
IFG→IFG/NFG	87	8	1.50 (0.72–3.15)	387	23	1.15 (0.69–1.93)
IFG→DM	62	8	2.47 (1.17–5.19)	101	12	2.14 (1.12–4.10)
DM	62	7	1.72 (0.78–3.80)	62	7	1.78 (0.79–4.00)

N/n: number of cases, NFG: normal fasting glucose, IFG: impaired fasting glucose, IFG→IFG/NFG: subjects classified as IFG at baseline and as IFG or NFG at follow-up, IFG→DM: subjects classified as IFG at baseline and as DM at follow-up, DM: diabetes mellitus type 2

0093

Physical inactivity and all-cause mortality in Australians with 'undiagnosed' impaired glucose metabolism. The AUSDIAB Study

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Background and Aims: Physical inactivity has been associated with increased all-cause mortality in the general population and in people with type 2 diabetes. However, little is known about the association of physical inactivity among people with 'undiagnosed' impaired glucose metabolism. The aim of this study was to examine the association between physical inactivity and all-cause mortality in Australian adults with impaired glucose metabolism.
Materials and Methods: The Australian Diabetes, Obesity and Lifestyle Study (AusDiab) is a nationally representative population-based survey of adults aged≥25 years. Baseline measurements were obtained between 1999 and 2000. Based on an OGTT, 2,471 individuals (1,238 men, 1,233 women) were identified as having impaired glucose metabolism (impaired fasting glycemia [IFG], impaired glucose tolerance [IGT], or undiagnosed type 2 diabetes) at baseline. Self-reported leisure-time physical activity status (physically inactive (0 mins/week) or physically active) was determined using interviewer-administered questionnaires. Potential confounders measured at baseline included: education, smoking status, diseases present at baseline (prior cardiovascular disease, hypertension, dyslipidaemia), waist circumference and television viewing time. Vital status was ascertained in 2005 from the National Death Index.
Results: Over a median follow-up of 5.2 years there were 131 deaths in those identified as having impaired glucose metabolism at baseline. Collectively, in those with impaired glucose metabolism, the age- and sex-adjusted odds ratio (OR) of all-cause mortality associated with being physically inactive was 2.10 (95% CI 1.40–3.15), which remained significant after adjustment for confounders — OR: 1.95 (95% CI 1.28–2.95). Among those with IGT and undiagnosed type 2 diabetes the odds ratios (adjusted for all confounders) of all-cause mortality associated with physical inactivity were 2.26 (95% CI 1.25–4.1) and 2.56 (95% CI 1.12–5.86) respectively. There was no association observed between all-cause mortality and physical inactivity in those with IFG — OR: 1.68 (95% CI 0.53–5.31).
Conclusion: These findings show that physically inactive adults with 'undiagnosed' impaired glucose metabolism have more than a two-fold increased risk of all-cause mortality over 5 years. These results further reinforce the importance of promoting physical activity in people with impaired glucose metabolism.

0094

Are patients with type 2 diabetes more likely to have diagnosed atherosclerosis? A large US epidemiologic study

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Background & Aim: Over last 2 decades, advances in cell and molecular biology have improved our understanding on the impact of atherosclerosis among patients with type 2 diabetes (T2D). However, up to now, no large US epidemiologic study has been conducted to compare atherosclerosis prevalence between patients with T2DM and patients without diabetes (NDM). Our study aims to fill this information gap through comparing age and gender specific atherosclerosis prevalence rates between patients with T2D and patients without diabetes (NDM) in a US primary care setting.
Methods: A retrospective cohort study was conducted based on a large national electronic medical records database that covered 4.2 million unique Americans in 2005. In the study, we reviewed medical records of 231,492 patients with T2D and a random sample of NDM patients (1,219,047). Based on our medical record review, we estimated the age and gender specific atherosclerosis prevalence rates for individual gender and age groups, as well as relative risks (RR) and odds ratios (OR) of having atherosclerosis between T2D and NDM patients.
Results: Table 1 presents age and gender specific atherosclerosis prevalence rates for T2D and NDM patients. On average, the atherosclerosis prevalence rates were 1.6% for patients with T2D, but 0.7% for NDM. The age and gender specific relative risks (or odds ratios) of having atherosclerosis between patients with T2D and those without ranged from 1.3 through 5.0 for female and from 1.1 through 2.3 for male with a possible inverse age trend.
Conclusions: Patients with T2D, on average, were twice likely to have atherosclerosis than patients without diabetes. The odds ratios of having atherosclerosis tend to be higher among females and among younger age groups. These results suggest the necessity of preventing, diagnosing and treating atherosclerosis aggressively among subjects with T2D.

Table 1: Age and Gender Specific Prevalence, Relative Risks and Odds Ratios of Atherosclerosis

Age Group (years)	Both	T2DM	NDM	Relative Risk*	Odds Ratios*
	Female				
20–29	0.1%	0.2%	0.0%	5.0	5.0
30–39	0.1%	0.2%	0.1%	4.7	4.7
40–49	0.2%	0.2%	0.2%	1.3	1.3
50–59	0.4%	0.7%	0.4%	1.9	1.9
60–69	1.0%	1.4%	0.8%	1.7	1.7
70–79	1.8%	2.4%	1.6%	1.6	1.6
80+	2.1%	2.9%	1.9%	1.6	1.6
All Ages	0.8%	1.6%	0.7%	2.3	2.3
	Male				
20–29	0.0%	0.0%	0.0%	–	–
30–39	0.1%	0.2%	0.1%	2.3	2.3
40–49	0.2%	0.3%	0.2%	1.1	1.1
50–59;	0.6%	0.8%	0.5%	1.7	1.7
60–69	1.2%	1.5%	1.1%	1.4	1.4
70–79	2.0%	2.6%	1.7%	1.5	1.5
80+	2.2%	3.0%	2.0%	1.5	1.5
All Ages	0.9%	1.6%	0.8%	2.1	2.1

Note: p<0.01 for all relative risks and odds ratios.

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0095

Population screening for type 2 diabetes and associated cardiovascular risk factors reduces mortality

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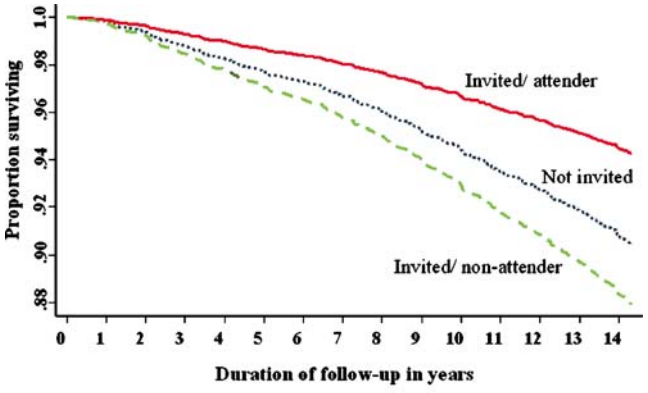
Background and Aims: Uncertainty remains over whether screening the general population for type 2 diabetes and associated cardiovascular risk factors will lead to overall health improvement. Prospective randomised trials of such screening programmes with mortality endpoints that might address this question will be expensive and take many years to complete.
Material and Methods: A parallel group population-based cohort study was undertaken in Ely, Cambridgeshire. In 1990 1706 people were randomly selected from a population of 4933 individuals aged 40 to 65 years, without known diabetes, and were invited for five-yearly screening for diabetes using the oral glucose tolerance test (OGTT). Results including values for cholesterol, triglyceride, glycosylated haemoglobin and blood pressure were forwarded to general practitioners without treatment recommendations. Mortality follow-up lasted 14.3 years.
Results: 68% of those invited attended the first round of screening, attendance was lowest among men (p=0.035) and those with greater material deprivation (Townsend index) (p=0.005). 108 participants had positive OGTTs during the three rounds of screening. A total of 581 participants died during follow-up, 245 cancer-related and 197 cardiovascular. Diabetes was recorded on 41 death certificates. After adjusting for age, gender and deprivation, invitation to screening was associated with a 20% reduction in overall mortality (hazard ratio: 0.80, 95%CI: 0.67 to 0.95). Compared to those not invited the hazard ratio for mortality was lower for those taking up the offer of screening (0.59, 95%CI: 0.47 to 0.73) and higher for those invited but not attending (1.28, 95%CI: 1.02 to 1.60).
Conclusion: Invitation to a screening programme for diabetes including assessment of cardiovascular risk factors appears to significantly reduce overall population mortality, although many at high risk did not attend. It is not possible to ascertain the mechanism underlying this observation from this retrospective study. Earlier detection of diabetes may partly account for these findings, but they may also be due to changes in patient and practitioner behaviour associated with the screening programme. We await the findings from prospective studies before advocating population screening.



Kaplan-Meier survival curves according to whether individuals were invited to screening and whether invited individuals attended screening (adjusted for age, gender and Townsend Index of deprivation)

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0096

Insufficient cardiovascular risk factor control following myocardial infarction in patients with type 2 diabetes: a register linkage study from the Swedish national diabetes register (NDR)

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Background and Aims: Cardiovascular risk factor control following myocardial infarction in patients with type 2 diabetes (DM2) is essential. The aim of this study was to examine the adherence to recent treatment guidelines in clinical practice.
Material and Methods: Patients with DM2 registered in the NDR (n=41475) were analysed for comparisons of cardiovascular risk factors between patients with and without previous myocardial infarction (MI), based on national register linkage analyses for hospital care (2003). The epidemiological definition of DM2 was: (i) therapy with diet alone or oral hypoglycaemic agents (OHAs), (ii) insulin in monotherapy or combined with OHAs in patients with an age >39 years at the onset of diabetes.
Results: The mean age of the patients with a previous myocardial infarction (n=4215; 32% women) were 72 years. The mean duration of diabetes in these patients was 11 years and the mean follow-up time after the MI was 9 years. The achievement of treatment goals of cardiovascular risk factors were 90% for non-smoking status, 65% for non-obesity, 64% for total cholesterol (TC) <5.0 mmol/L, 56% for both HbA1c <7.3% and triglycerides <2.0 mmol/L, and 34% for blood pressure (BP) <130/85 mmHg. These frequencies, compared to patients without previous MI (n=32498), were considerably higher concerning BP and TC, and somewhat higher regarding HbA1c, non-obesity and non-smoking.
Treatment with antihypertensive– (34% vs. 19%) and lipid-lowering drugs (70% vs. 58%) and aspirin (80 vs 33%) were considerably higher in patients with a previous MI. The DM2 patients with a previous MI who had reached the treatment targets, were more oftenly treated with diet (88–75%) and OHAs (62–60%), but not with insulin, for the achieved HbA1c level, than the DM2 patients without a previous MI. Patients with a longer follow-up period after the MI than 9 years had, compared to patients with a shorter follow-up period, a tendency to higher achievement of the HbA1c target in 2003, but lower achievement of the TC level.
A logistic regression analysis of a subgroup of 33586 DM2 patients (with complete data for all variables in 2003) showed that a previous MI (dependent variable) was independently associated with hyperlipidaemia (untreated with TC>5.0 mmol/L or triglycerides>1.7 mmol/L, or treated), odds ratio (OR) 2.89 (p<0.001), and with hypertension (untreated with BP>140/90 mmHg, or treat ed), OR 2.49 (p<0.001). The association was less evident for HbA1c >8.3%, OR 1.24 (p<0.001), and with smoking, OR 1.13 (p<0.05).
Conclusion: Although risk factor treatment seems to be intensified in type 2 diabetic patients, who have suffered a MI, the degree of fulfilment of the treatment goals is still insufficient in this high-risk group of patients.

OP 17

Mechanisms of beta cell dysfunction and death

0097

Pdx-1-driven differentiation-dependent sensitivity to interleukin-1 toxicity in insulin-producing INS1rαβ cells is related to enhanced JNK and NFκB activity and a distinct gene expression profile

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Background and Aims: Pancreatic β-cells are more sensitive to several toxins (e.g. streptozotocin, alloxan, and cytokines) than the other endocrine cell types in the islets of Langerhans. We have previously shown that β-cell acquisition of sensitivity to interleukin (IL)-1 induced apoptosis is differentiation-dependent and associated with pancreatic duodenal homeobox factor-1 (Pdx-1) expression. Pdx-1 is an important transcription factor regulating pancreatic development and islet cell function, but the molecular mechanisms by which Pdx-1 leads to enhanced cytokine-sensitivity are currently unknown. *Rationale:* Cytokines secreted by infiltrating inflammatory cells have been implicated in 1) islet allograft rejection, 2) disease recurrence in isografts and 3) non-specific inflammation caused by transplantation trauma even when using islet microencapsulation. It is therefore important to understand the molecular actions of cytokines on the β-cell as well as differentiation-dependent steps causing the particular sensitivity of pancreatic β-cells to inflammatory cytokines compared to non-β-cells. *Aims:* (1) To compare IL-1-induced apoptosis and signaling mechanisms in INS-1rαβ cells+/− Pdx-1 overexpression. (2) To clarify gene expression profiling and transcription

factor networks of differentiation-aquired β-cell-sensitivity to cytokines providing a molecular fingerprint of inflammatory sensitivity.

Materials and Methods: Doxycycline-inducible Pdx-1 expression in INS-1rαβ was used as a model of β-cell maturation. IL-1-induced cell death was determined by TUNEL, cleavage of caspase-3 and ELISA detection of histone-DNA complexes in the cytoplasm. Activation of MAPKs was determined by Western blot analysis using phosphospecific antibodies and nuclear translocation of NFκB was determined by electric mobility shift assay (EMSA) and Luciferase reporter assay. Affymetrix microarray analysis was performed to investigate the molecular mechanisms of enhanced IL-1 sensitivity.

Results: Pdx-1 over-expressing INS-1rαβ cells were more sensitive (310%, p<0,05) to the apoptotic effect of IL-1 as determined by TUNEL, cytoplasmic histone-DNA complexes and a distinct IL-1-induced cleavage of caspase-3. IL-1-induced JNK activity determined by phosphorylation was markedly enhanced and prolonged in Pdx-1 overexpressing INS-1rαβ cells. Furthermore, IL-1-induced IκB degradation was increased correlating with augmented NFκB binding to DNA κB sites and a>2 fold increase in NFκB transcriptional activity in Pdx-1 overexpressing cells. These observations were associated with complete separation of the expression of gene clusters in response to IL-1 depending upon Pdx-1 overexpression, indicating that Pdx-expression distinctly modifies IL-1 induced signaling pathways and transcription factor networks.

Conclusion: Our data suggest that the final β-cell maturation accompanied by increased IL-1 sensitivity depend upon the expression of genes regulated by Pdx-1 leading to enhanced and prolonged JNK and NFκ¢ activity.

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0098

Molecular pathways underlying Toll-like receptor 3 (TLR3)-induced beta cell apoptosis

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Background and Aims: The mechanisms by which viruses, or viral products such as double-stranded RNA (dsRNA), affect beta cell function and survival and trigger autoimmunity in type 1 diabetes remain unknown. We have shown that dsRNA, in association with IFN-γ, triggers beta cell apoptosis. By microarray analysis of human islets and FACS-purified rat beta cells we observed that infection by the Coxsackievirus B5 or treatment with dsRNA+IFN-γ up-regulates the expression of type I interferons and other genes contributing for islet inflammation and beta cell death. Several of these viral/dsRNA-induced genes are potentially regulated by the Toll-like receptor 3 (TLR3), which is expressed and apparently functional in beta cells. We presently investigated the pro-apoptotic signaling pathway downstream of TLR3 in beta cells.

Materials and Methods: FACS-purified rat beta cells and dispersed islet cells from wild type (wt) or $TLR3^{-/-}$ mice were cultured for 3 to 5 days, with or without synthetic dsRNA (PIC; 1–100 μg/ml; transfected or not with lipofectamine), IFN-γ (500 U/ml) and/or IL-1β (50 U/ml). Viability was determined by the nuclear dyes HO 342 and propidium iodide, while gene expression was determined by real-time RT–PCR. Activation of NF-κB and IRF-3 was studied respectively by immunocytochemistry and Western blot using an antibody specific for the phosphorylated form of IRF-3.

Results: Exposure of dispersed islet cells from wt mice for 5 days to PIC (100 μg/ml)+IFN-γ, but not to PIC alone, increased apoptosis (47% vs 21% in control wt cells; p<0.005; n=5). On the other hand, islet cells from $TLR3^{-/-}$ mice were completely resistant to dsRNA+IFN-γ-induced apoptosis (18% vs 17% in control $TLR3^{-/-}$ cells; n=5). PIC induced NF-κB and IRF-3 activation in wt but not in $TLR3^{-/-}$ islet cells. These data suggest a key role for TLR3 in beta cell apoptosis triggered by exogenous dsRNA. During viral infection beta cells are exposed to both extra- and intracellular dsRNA, and introduction of foreign dsRNA (PIC; 1 μg/ml) by transfection into islet cells induced similar degree of apoptosis in wt and $TLR3^{-/-}$ islet cells. This suggests that TLR-3-independent mechanisms are involved in the intracellular recognition of dsRNA. Exogenously added or transfected dsRNA up-regulated TLR3 and PKR mRNAs to a similar extent and did not affect iNOS expression in rat beta cells. Internal dsRNA, however, induced a 50–100 fold higher up-regulation of IFN-α and IFN-β mRNAs. We have previously shown that dsRNA+IFN-β induces beta cell apoptosis.

Conclusions: The TLR3 plays a crucial role in exogenous dsRNA-induced NF-κB and IRF-3 activation and dsRNA+IFN-γ-induced apoptosis. Introduction of foreign dsRNA into pancreatic beta cells triggers apoptosis by a TLR3- and NO-independent manner. Increased production of type I interferons probably contributes for this effect.

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0099

Transgenic overexpression of hormone-sensitive lipase in immortalised mouse β-cells rescues the cells from lipotoxicity

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Background and Aims: Lipid perturbation and accumulation of triglycerides in pancreatic β-cells are associated with impaired insulin secretion. Hormone-sensitive lipase (HSL) is expressed and active in β-cells and may be involved in regulating the turn-over of the triglyceride pool. The aim of the study was to create a stable mouse pancreatic β-cell line overexpressing HSL to evaluate the ability of these β cells to retain GSIS after exposure to fatty acids in the presence of low and high glucose levels.
Materials and Methods: A mouse strain carrying an HSL transgene under the control of the insulin promoter was crossed with a mouse strain developing spontaneous insulinomas (RIP SV40). Tumour cells from offspring containing the transgene were used to establish a β cell line with a stable overexpression of HSL (tgHSL-β) together with a control cell line that did not carry the transgene (wt-β).
The two cell lines were exposed to glucose or glucose in combination with palmitate for 72 h, after which glucose responsiveness was assessed by measuring insulin release during 60 min in response to 1.0, 11.1 or 16.7 mM glucose. Viable count was assessed after 72 h using a commercially available MTS assay. Palmitate oxidation and triglyceride (TG) content in cells were analysed after 72 h exposure to 0.5 mM palmitate in 3.3 or 16.7 mM glucose.
Results: The number of viable cells was increased in tgHSL-β compared to wt-β cells when cultivated for 72 h in 16.7 mM glucose (191% viable tgHSL-β cells and 150% viable wt-β cells). Exposure of the β cell lines to 0.25 mM palmitate and 16.7 mM glucose for 72 h decreased wt-β cell viable count to 65%, whereas tgHSL-β retained the same number of viable cells (116%) as with 3.3 mM glucose.
TG content in tgHSL-β and wt-β cells was equal after 72 h exposure to 3.3 mM and 16.7 mM glucose. Exposure for 72 h to 0.5 mM palmitate and 3.3 or 16.7 mM glucose the TG content was increased to 50/56 μg TG/mg protein in wt-β cells but remained 19/16 μg TG/mg protein in tgHSL-β cells. Palmitate oxidation in either of the cell lines was not increased by incubation for 72 h with palmitate disregarding glucose level.
Insulin content was increased to 205% in tgHSL-β compared to in wt-β cells when cultured in 3.3 mM glucose. After exposure to 0.5 mM palmitate, the insulin content in the two cell lines was found to be equal (0.23 and 0.25 μg insulin/mg protein respectively).
At acute exposure to glucose and GLP-1, carbacholin or forskolin insulin secretion was improved in tgHSL-β compared to the wt-β cell line at glucose concentrations over 8.3 mM. Acute exposure to palmitate lead to higher insulin secretion in wt-β than in tgHSL-β cells at low but not at higher glucose levels. Chronic (72 h) exposure to 0.5 mM palmitate at high glucose concentration (16.7 mM) resulted in intact glucose responsiveness in tgHSL-β cells although insulin secretion was increased compared to tgHSL-β cells without palmitate. However, the wt-β cell line displayed blunted glucose responsiveness after 72 h of palmitate exposure, also with an overall increase in insulin secretion.
Conclusion: The β cell line overexpressing HSL displays resistance to conditions of high glucose as well as high palmitate levels compared to the wildtype β cell line, manifested both as a higher maintained proliferation rate and a retained GSIS function after long time exposure to high levels of glucose and palmitate.

0100

Unfolded protein response in fatty acid treated pancreatic β-cells

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Background and Aims: The unfolded protein response (UPR) is a physiological reaction elicited by conditions of endoplasmic reticulum (ER) -stress such as accumulation of unfolded, misfolded or excessive amounts of proteins in the ER. The UPR coordinates attenuation of general protein synthesis, increased capacity of protein folding through up-regulation chaperones and enhancement of protein degradation with the aim to relieve the cell from the ER-stress. Failure to cope with ER-stress can trigger apoptotic cell death. When either primary β-cells or insulin-producing cell lines are cultured with free fatty acids for long time periods apoptosis is increased. Recently it was found that palmitate and oleate increased mRNA levels of some ER-stress markers indicating that free fatty acids can induce ER-stress. The aim of the present study was to investigate the activity and timing of the UPR pathways in response to fatty acid treatment.
Materials and Methods: The insulin-producing cell lines INS-1E and MIN6 were exposed for 0,2,4,8,12 and 24 hours to palmitate (0.5 mM), oleate (0.5 mM) or palmitate

in combination with diazoxide (250 μM). In addition, cells were exposed to thapsigargin (300 nM), an ER-stress inducer. After exposure, the cells were lysed and key proteins of the PERK and ATF6 signaling pathways of UPR were measured by western blotting.

Results: To analyse the activity in the PERK pathway we measured phosphorylation of eukaryotic initiation factor 2α (eIF2α) and the cellular levels of the apoptotic protein CHOP. In INS-1E cells palmitate caused a 2.5-fold increase of eIF2α phosphorylation already after 2 hours of exposure. The degree of phosphorylation continued to rise over the study period reaching a 10-fold increase after 24 hours. In MIN6 cells palmitate triggered a kinetically similar response although the maximal induction of eIF2α phosphorylation was only 2-fold in these cells. Oleate did not induce phosphorylation of eIF2α in either INS-1E or MIN6 cells. The degree of eIF2α phosphorylation in cells cultured in the presence of palmitate was reduced by 40–60% when diazoxide was included in the culture medium. Thapsigargin induced a prompt (after 2 hours) and sustained elevation of eIF2α phosphorylation in both INS-1E and MIN6 cells. Palmitate also increased CHOP levels but at a later (6 h) stage than eIF2α phosphorylation. In INS-1E and MIN6 cells the cellular level of CHOP protein began to rise after 8 hours and after 24 hours a more than 4-fold increase was observed. The induction of CHOP protein expression by palmitate was counteracted by inclusion of diazoxide in the culture medium. No rise in CHOP was observed in cells exposed to oleate. Elevated levels of CHOP were also evident in thapsigargin treated cells. To analyse the activity in the ATF6 pathway we measured the cellular levels of the chaperone proteins GRP78/BiP, PDI and PPI-B. None of the chaperone proteins demonstrated elevated levels in cells exposed to either palmitate, oleate or thapisgargin.

Conclusion: Palmitate and thapsigargin, but not oleate, preferentially activate the PERK pathway of the UPR. Since phosphorylation of eIF2α negatively regulates protein synthesis our results (sustained elevation of eIF2α phosphorylation) could provide an explanation for the inability of pancreatic β-cells to biosynthetically compensate enhanced basal insulin secretion caused by fatty acids. This in turn would lead to a reduced insulin content.

0101

Beta cell damage in nutritionally-induced T2DM in the gerbil *Psammomys obesus* (sand rat) is not cytokine mediated

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Background and Aims: *Psammomys obesus* is a well established animal model for type 2 diabetes mellitus (T2DM) developing hyperglycaemia, initially hyperinsulinaemia and later hypoinsulinaemia accompanied by insulin resistance when placed for one to three weeks on a high energy (HE) diet. Recently it has been proposed that cytokines may be involved also in the aetiopathology of T2DM. Therefore a morphological and biochemical analysis using either isolated pancreatic islets or fixed pancreatic sections during the time course of islet destruction was performed to evaluate changes in the cytokine and enzyme expression in pancreatic beta cells in the time interval from one to three weeks on HE diet in comparison to the control situation.

Materials and Methods: The gene expression of the proinflammatory cytokines IL-1β, IFN-γ and TNF-α and of the IL-1β induced enzyme iNOS was analyzed using gerbil specific primers by *quantitative* real time RT–PCR and by *in situ* RT–PCR, whereas for insulin and the enzyme caspase 3 expression analysis rat primers were used because these gerbil gene sequences are unknown. To detect the protein of these cytokines and enzymes antibodies were used recognizing human, rat and mouse specific protein sequences because gerbil specific antibodies are not commercially available.

Results: The studies on gene expression by *in situ* and *q*RT–PCR showed no mRNA expression for IFN-γ, TNF-α and iNOS as well as caspase 3 during diabetes development in the sand rat at different stages of islet destruction. In comparison to pancreas draining lymph nodes a more than 10-fold lower gene expression of IL-1β was observed in isolated control islets, which was not induced by hyperglycemia for 7 or 21 days during diabetes development. Pancreatic beta cells both in the normoglycaemic as well as hyperglycaemic situation remained immunohistochemically unstained for all proinflammatory cytokines, iNOS and caspase 3 whereas the insulin staining on the protein and the gene expression level decreased stepwise from day 7 to day 21 on HE diet. The small numbers of immune cells, only macrophages and neutrophils, showed also no gene and protein expression for proinflammatory cytokines and iNOS in pancreatic islets of normoglycaemic and hyperglycaemic animals. All proinflammatory cytokines were detectable on the gene and protein level in the lymphoid tissue of both the pancreas draining lymph nodes and the spleen in the control and the diabetic situation.

Conclusion: Thus it can be concluded that the mechanisms leading to beta cell damage during the development of hyperglycaemia in the sand rat on HE diet were not mediated by induction of proinflammatory cytokines and a resulting upregulation of iNOS induced by IL-1β. Caspase 3 as the first executor caspase leading to apoptotic cell death remained undetectable confirming that the intra-

cytoplasmic vacuoles appearing in beta cells on HE diet are a sign of a necrotic cell damage is this T2DM animal model.

0102

Bcl-2 acutely regulates β-cell Ca^{2+} signaling and apoptosis via IP_3R- and RyR-mediated mechanisms

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Background and Aims: Members of the Bcl-2 family of proteins are well known regulators of β-cell death, and the diabetic state is associated with an increased ratio of pro-apoptotic to anti-apoptotic members in the pancreatic islet. However, to date little is known about the acute physiological functions played by endogenously expressed anti-apoptotic Bcl-2 proteins. The aim of the present study was to examine the moment-to-moment role of Bcl-2 in the regulation of β-cell function and survival, with emphasis on its putative functional interaction with intracellular Ca^{2+} release channels and β-cell Ca^{2+} homeostasis.
Materials and Methods: We used the cell-permeable small molecule Bcl-2 inhibitors *HA14-1* and *Bcl-2 inhibitor* (Bcl-2i) to acutely inhibit anti-apoptotic Bcl-2 in primary mouse islet cells and MIN6 cells. The effect of various doses of the inhibitors was examined alone or in combination with modulators of cellular Ca^{2+} homeostasis. Cell death was quantified in real-time over a period of up to 48 hours by monitoring cellular propidium Iodide (PI) incorporation using an automated Cellomics KineticScan™ imaging system. The involvement of cell death pathways was investigated by Western blotting, and the results quantified by band densitometry. Caspase-3 activation was also monitored by real-time microscopy as the loss of FRET between cyan- and orange-emitting fluorescent proteins linked by a peptide containing the 'DEVD' caspase-3 cleavage sequence. Changes in cytosolic Ca^{2+} were recorded using fura-2.
Results: Inhibition of endogenously expressed Bcl-2 proteins, by HA14-1 or Bcl-2i, induced caspase-3 activation and cell death of MIN6 and primary β-cells. The onset of β-cell death was rapid in response to 20 μM Bcl-2i, with PI incorporation and caspase-3 activation occurring between 2 and 5 hours after treatment. Cell death induced by Bcl-2i and HA14-1 was not associated with activation of the ER stress, as indicated by normal levels of CHOP/GADD153 expression after 24 hours exposure to 20 μM and 100 μM of the inhibitors. In primary β-cells both Bcl-2i and HA14-1 stimulated rapid cytosolic Ca^{2+} fluctuations, followed by a slow rise to sustained supraphysiological Ca^{2+} levels, and eventually loss of plasma membrane integrity. The first phase of Bcl-2i-induced fluctuations was rapidly reversible at all doses tested. Cell death was significantly reduced after pre-treatment with 50 μM BAPTA or inhibition of intracellular IP_3R and RyR Ca^{2+} release channels with 1 μM xestospongin C and 100 μM ryanodine, respectively. Moreover, xestospongin C and ryanodine both lowered the amount of cleaved caspase-3 detected at 2 hours by approximately 50%.
Conclusion: Acute inhibition of Bcl-2 rapidly activates caspase-3 and evokes Ca^{2+}-regulated β-cell death. Moreover, our findings provide evidence for a moment-to-moment role for endogenous Bcl-2 in regulation of β-cell calcium homeostasis. Specifically, inhibitor-induced caspase-3 activation and apoptosis depends on flux through IP_3 and ryanodine receptors. Together, these results suggest that Bcl-2 regulates β-cell ER Ca^{2+} homeostasis and exerts its anti-apoptotic effect in the β-cell by controlling Ca^{2+}-mediated signaling between ER and mitochondria.
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OP 18

Diabetes in pregnancy

0103

Following in Mom's footsteps? Preliminary assessment of the health and lifestyle status of teenage girls born to gestational diabetic women

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Background and Aims: Gestational Diabetes (GDM) appears to increase the risk of obesity, type 2 Diabetes Mellitus (DM) and the metabolic syndrome in women and their offspring. This study seeks to determine health and lifestyle risk factors that might make daughters of GDM women more vulnerable to diabetes and its associated conditions.
Study Design: This is a longitudinal follow-up of a case-control cohort of women that delivered a baby girl between 1989 and 1991 at the Royal Victoria Hospital. Consenting mother–daughter pairs were assessed for anthropometric, behavioral (diet, physical activity) and metabolic (glucose intolerance, insulin resistance, dyslipidemia) and other clinical and novel indicators.
Results: A double entered and edited database of 103 (48 case and 55 control) mother–daughter pairs was available for preliminary analyses. Characteristics of case (GDM) and control (Non-GDM) moms and daughters are presented in Table 1. To date, no daughters of cases or controls have

type 2 DM, impaired fasting glucose (IFG) or impaired glucose tolerance (IGT). HOMA $_{IR}$ was significantly elevated in daughters of women that either had GDM or glucose intolerance during their pregnancy, however the difference was not significant in linear regression analyses controlling for daughters' waist circumference. Case daughters, as a group, were non-obese but tended to have a higher body mass index (BMI) and were less active (in MET scores). As for the mothers, fasting glucose and 2 hr OGTT results indicate that 17 (37%) cases are now diabetic, and that an additional 6 (13%) either have IFG, IGT or both IFG and IGT. In contrast, 2 (3.6%) control mothers have diabetes, 7 (12.7%) have IFG and one (1.8%) has both IGT and IFG. As expected, women that were glucose intolerant during their pregnancy were significantly heavier, had a worse lipid profile, were found to be more insulin resistant and tended to be less active than women with a history of glucose tolerant pregnancies (Table 1). In the available data,WC measures were highly correlated between mothers and daughters (r=0.39, p<0.0001), whether they were cases or controls. Mother's GDM case status was an important and statistically significant determinant of daughters' waist circumference (*p*-values <0.01). The saturated fat intake and leisure time physical activity in MET scores of the mothers was positively and significantly correlated with their daughter's saturated fat intake as a percent of total energy (*r*=0.42, p<0.0001) and daughter's MET scores (*r*=0.24, p=0.017).

Table 1– Characteristics of Case (GDM) and Control (Non-GDM)

	DAUGHTERS			MOTHERS		
Characteristics	GDM	Non-GDM	p-value	GDM	Non-GDM	p-value
Age (years)	14.6±0.7	15.1±0.6	<0.001	46.9±4.0	46.8±4.8	N.S.
BMI (kg/m2)	23.5±4.6	22.1±3.3	0.07	29.4±6.2	26.4±4.5	<0.01
Waist Circumference (cm)	79.9±10.8	74.9±8.3	<0.01	95.6±14.1	87.8±12.3	<0.01
Body Fat %	30.6±6.7	28.8±7.0	N.S.	37.9±7.2	34.3±7.3	<0.01
HDL Cholesterol	1.2±0.3	1.2±0.2	N.S.	1.2±0.3	1.5±0.4	<0.01
Total Cholesterol (mmol/L)	4.0±0.6	4.3±0.7	0.07	5.4±1.0	5.3±1.3	N.S.
Triglycerides	0.7±0.3	0.7±0.4	N.S.	1.5±1.2	1.1±0.9	0.05
HOMA–IR	2.2±1.3	1.7±0.7	<0.01	3.4±3.6	1.5±1.0	<0.001
MET (hrs/week)	60.6±37.1	74.2±49.2	0.09	18.0±22.7	26.1±22.1	0.07
Saturated Fat Intake (% total energy)	11.1±2.8	11.2±2.1	N.S.	11.2±2.8	11.3±2.5	N.S.

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Conclusion: Although teenage girls of GDM cases in our population appeared to be healthier than similar offspring of other study populations, maternal GDM was an important determinant of daughters' BMI, central fat patterning and insulin resistance levels. Shared lifestyle factors such as low physical activity levels may be aggravating risks of diabetes in offspring of pregnancies affected by GDM.

0104

Insulin sensitivity and beta cell function in pregnancy

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Background and Aims: Uncomplicated pregnancy is characterized by insulin resistance and increased insulin secretion. We retain of interest to evaluate insulin sensitivity and secretion in pregnant women with normal glucose tolerance and gestational diabetes, in the early and late period of pregnancy by utilizing as fasting indices of insulin sensitivity IS_{QUICKI} and as OGTT index of insulin action OGIS as insulin secretion the disposition index (DI)
Materials and Methods: 922 pregnant women among which 829 with normal glucose tolerance (NPW) (mean age 31±4 years, mean BMI 22.5±3) and 93 GDM patients (mean age 33±4 years, mean BMI 23±3) were evaluated in early (16–20 g.w.)and in late pregnancy (26–30 g. w). Plasma glucose and insulinemia levels were evaluated in fasting state and after 1 and 2 hours og OGTT (75 gr glucose). As for sensitivity index we calculated $IS_{-QUICKI}$ and OGIS. Insulin secretion was evaluated with the area under the insulin concentration curve (AUC), beta cell function: with the ratio of the insulin AUC to glucose AUC for the whole test period (insulinogenic index).The parameter that describes this relationship is the disposition index (DI), calculated as OGIS times the insulinogenic index. DI quantifies the ability of the beta cell to compensate for reduced insulin sensitivity by increasing its secretion.
Results: In early stage GDM women with respect to NPW had higher levels of fasting plasma glucose (FPG 74±7 mg/dl vs 78±8 mg/dl;p<0,001) and comparable insulin (FPI 6.4±6.7 μU/ml vs 6.2±4.0) ;AUC under the respective curves was significantly (p<0.00001)elevated in GDM (AUC glucose 12.1±2.2 vs 15.1±2.9 and AUC insulin

4.7±2.3 vs 5.9±2.9 respectively)QUICKI (0,40±0.06 vs 0.39±0.06) are not different, while periferal insulin sensitivity OGIS was significantly lower in GDM(468±68 vs 427±58;p<0.001).insulin secretion in basal condition and dynamic B-cell function were similar in both groups.In Late pregnancy GDM women show higher FPG(p<0.0004),not different FPI,dynamic AUC higher significantly and both liver and periferal insulin sensitivity lower (QUICKI 0.39±0.06 vs 0.37±0.04;p<0.05;OGIS 432±60 vs 396±57; p<0.0001) No difference was detected in B-cell function. During pregnancy, from early to late period, in both group FPG, FPI increased as well as AUC,QUICKI and OGIS instead were reduced.B-cell function increased in NPW (17%,p<0.00001) but less in GDM(13%,p0.03). Disposition index ,not different in early period, show in late pregnancy an overcompensation in NPW ,lower in GDM (p0.02) showing an inability of GDM women B-cell to relase more hormone to augment glucose disposal.

Conclusion: Our data, on a large number of cases, show that insulin sensitivity decreases in pregnancy, but in early pregnancy only OGIS already predicts the deterioration in metabolic status that GDM encounter later on .Beta cell function is reduced and the DI is less able than betac cell function to respond to physiological insulin resistance in GDM.

0105

Increased levels of circulating adhesion molecules (ICAM, VCAM) in women with previous gestational diabetes

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Background and Aim: Gestational Diabetes (GD) can serve as a model of type 2 diabetes (T2DM). Women with prior GD often feature insulin resistance and are at increased risk of premature atherosclerosis. Therefore we investigated in women with a history of GD in comparison to women with normal glucose tolerance during and after pregnancy (controls) surrogate parameters of inflammation and endothelial dysfunction.

Material and Methods: 119 women with previous GD (Age: 33.76±0.73 years, BMI: 27.65±0.51 kg/m^2) and 41 control women (Age: 31.55±0.88 years, BMI: 25.01±0.89 kg/m^2) underwent an oral (75 g; OGTT) and an intravenous (FSIGT) glucose tolerance test 14–16 weeks after delivery. The women with prior GD were reinvestigated by an OGTT after one year. At both visits no woman had any sign of infection and plasma concentrations of the circulating adhesion molecules vascular cell adhesion molecule (VCAM), intercellular adhesion molecule-1 (ICAM-1) and E-selectin (ELAM), plasma endothelin and leptin as well as inflammatory markers (sCRP, IL-6, fibrinogen, PAI-1) were measured. Insulin sensitivity (S_I) was calculated from the insulin modified FSIGTs.

Results: At baseline women with a history of GD showed higher concentrations of ICAMs (298.5±7.36 ng/ml; p<0.0001) and VCAMs (611.8±18.71 ng/ml; p<0.003) but not E-Selectin or endothelin compared to the control women (ICAM: 238.0±8.67 ng/ml, VCAM: 502.3±26.31 ng/ml, respectively). In addition sCRP (p<0.05) and PAI-1 (p=0.01) were higher and S_I (p<0.001) was lower in patients with prior GD than in the control women. S_I was inversely related with ELAM (r=−0.21; p=0.01), VCAM (r=−0.23; p<0.01) and ICAM-1 (r=−0.22; p<0.01) as well as with sCRP (r=−0.52; p<0.0001), IL-6 (r=−0.25; p=0.01), and fibrinogen (r=−0.24; p=0.003). Furthermore ICAM (r=0.22; p<0.01) and VCAM (r=0.18; p=0.02) were associated with sCRP, while ICAM and ELAM correlated with fibrinogen concentrations. All adhesion molecules also related to plasma leptin (r=0.17; p<0.02) and BMI (r=0.18; p<0.01). At one year follow up ELAM (p<0.0007) decreased in women with prior GD, while ICAM-1 and VCAM did not change significantly. Furthermore plasma leptin (p<0.02) increased, but all other markers of inflammation or endothelial dysfunction and also BMI remained unchanged at the follow-up visit.

Conclusion: Women with a history of GD are characterised by higher plasma levels of adhesion molecules which relate to insulin resistance, inflammatory parameters and plasma leptin and the degree of obesity. Whether these persistingly increased levels of ICAMs and VCAMs can predict development of a premature atherosclerosis in women with a history of GD remains to be determined.

0106

Intensive management of pregestational diabetes mellitus in pregnancy: Comparison of outcomes of type 2 diabetes with type 1 diabetes

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Background: The incidence of T2DM in the reproductive years is increasing. Previous data suggests that outcomes in

T2DM in pregnancy are poor. There is little information on the impact of intensive clinical management on pregnancy outcomes in T2DM.

Materials and Methods: We present data on 67 women with T2DM in pregnancy and 372 women with T1DM who attended at the Combined Diabetes/Obstetrical Clinics in three university maternity hospitals in Dublin, Ireland from 1995–2005. Diabetes care at each clinic was delivered by the same endocrinologists (RF, BTK, MMB). All women were treated intensively with insulin from time of booking at clinic. Management and glycaemic goals were identical for T1DM and T2DM groups.

Results:

	T2DM (n=67)	T1DM (n=372)	P value
Age (yrs)	34 0±5	30±5	<0.001
DM Duration (yrs)	3.3±2	13.0±8	<0.001
Baseline Wt (kg)	88±17	72±12	<0.001
Booking Gest (Wks)	9.8±5	8.4±5	<0.05
Hba1c %–Booking	7.3±1.6	7.7±1.5	0.06
13 weeks	6.2±1.0	6.6±1.0	0.01
26 weeks	5.4±0.8	6.6±0.8	<0.001
Term (>36 weeks)	5.9±0.9	6.4±0.3	<0.001
Birth Weight (Kg)	3.4±0.8	3.6±0.7	0.01

First trimester miscarriage rates were similar in T2DM (13/67) 19% and T1DM (70/372) 18%. Perinatal mortality rate in the T2DM group was 55/1000 (3/54) vs 36/1000 (11/302) total births in the T1DM group. Major congenital malformation rate (CMR) was 93/1000 (5/54) vs 37/1000 (14/302) total births for T1DM. Lethal CMR was 55/1000 in T2DM vs 10/1000 in T1DM. All 3 lethal CMR in T2DM were congenital cardiac defects.

Conclusion: Compared to women with T1DM in pregnancy, women with T2DM are older, heavier, have shorter DM duration, book later to a specialist clinic, achieve better glycaemic control and have lower infant birth weights. However T2DM in pregnancy is associated with higher rates of major congenital malformations, especially congenital cardiac defects. Women of reproductive age with T2DM should be identified for intensive therapy and education pre-pregnancy in an attempt to improve outcomes.

0107

Interaction between maternal type 1 diabetes and HLA DR4 increases fetal growth

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Background and Aims: Maternal diabetes and genetic factors affect intrauterine growth. The aim of this study was to determine the interaction between maternal diabetes, birth weight and type 1 diabetes susceptibility genes.

Materials and Methods: Birth weight and gestational age were obtained in newborn children of mothers with type 1 diabetes (n=1191), fathers or siblings with type 1 diabetes (n=917), and mothers with gestational diabetes (n=619). Birth weight, weight and height at age 1, 2 and 5 years were obtained from pediatric records and expressed as percentiles of national reference data corrected for age and sex. All 2108 children with mother, father or sibling with T1DM had HLA DR typing, and 1120 of these children also had INS VNTR typing.

Results: The median birth weight in children of mothers with T1DM was at the 69th percentile (IQR, 36th–91th percentile; P<0.0001) and in offspring of mothers with GDM was at the 64th percentile (IQR, 35th–90th percentile; P<0.0001) of the normative population. Fetal growth was influenced by strong interactions between maternal type 1 diabetes, sub-optimal metabolic control and the presence of the HLA DR4 allele in the child. Birth weight increased with moderately elevated HbA1c (5.5%–6.5%: median, 74th percentile; IQR, 47th–91st percentile; P6.5% (median, 86th percentile; IQR, 65th to 96th percentile, P<0.0001). In children of mothers with type 1 diabetes, birth weight was significantly increased if children had the HLA DR4 allele (75th vs 63rd percentile; P<0.0001). No differences were observed with other HLA alleles. HLA DR4 did not influence birth weight in children of non–diabetic mothers. Multivariate analysis indicated that HLA–DR4 in the child (P=0.001) contributed to birth weight independently from maternal HbA1c during pregnancy (P<0.0001), and parity status (P6.5% during pregnancy had the highest proportion of overweight children (23% at age 5 years).

Conclusion: A diabetic intrauterine environment interacts with gene(s) marked by the type 1 diabetes susceptibility HLA DR4 allele to increase fetal growth.

0108

Preeclampsia – a predictor of diabetic nephropathy?

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Background and Aims: A preeclamptic pregnancy is associated with a substantially increased risk of cardiovascular disease later in life in non-diabetic women. It is also well known that type 1 diabetes increases the risk of preeclampsia (PE) and pregnancy-induced hypertension (PIH). However, it is not known whether PE or PIH increase the risk of diabetic nephropathy (DN) later in life. The aim was to study whether PE and PIH are predictors of DN in type 1 diabetic patients in a prospective cross-sectional setting.
Materials and Methods: A total of 203 type 1 diabetic women, who were pregnant during the time period 1988–1996 and followed at the Department of Obstetrics and Gynecology in Helsinki, were re-studied after a median of 10.6 years within the nationwide, multi-center Finnish Diabetic Nephropathy Study (FinnDiane). Patients with proteinuria in the two first trimesters (Whites class F) or prepregnancy hypertension were excluded from the nephropathy analyses. So far, we have been able to verify (at least three recent urine collections) the renal status of 180 patients. Further characterization is ongoing. Incident DN was defined as microalbuminuria, macroalbuminuria or end stage renal disease (ESRD).
PIH was defined as an increase in the diastolic blood pressure of=15 mmHg or the final level=90 mmHg after 20 weeks of gestation. If proteinuria (=0.3 g/24 h) was detected after 20 weeks, the patient fulfilled the criteria for PE. At the FinnDiane visit, data on micro- and macrovascular complications as well as phenotypic characteristics were collected. SPSS 12.0.1 for Windows was used to analyze categorical variables with the χ^2 -test and continuous variables with Student's *t*-test. A logistic regression analysis with DN as the dependent variable was performed.
Results: Out of 180 patients 43 had had PE, 32 PIH and 105 a non-hypertensive pregnancy. A total of 38 patients had developed DN (2 ESRD, 9 macroalbuminuria, 27 microalbuminuria). Patients with prior PE had more often DN (DN vs normal albumin excretion rate (AER); 41.9% vs 8.9%, $P<0.001$). Patients with a history of PIH did not have more DN (10.3% vs 8.9%; $P=0.81$). Coronary heart disease was more prevalent in patients with a history of PE (12.2% vs. 2.2, $P=0.03$) In the logistic regression analysis PE ($P<0.001$) and HbA_{1c} ($P<0.001$) were independently associated with incident DN in a model that further included follow-up time, BMI, smoking, diabetes duration and age.
Conclusion: These preliminary data suggest that preeclampsia (PE), but not pregnancy induced hypertension (PIH), may be considered a predictor of incident diabetic nephropathy. PE predicts also CHD in type 1 diabetic patients.
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OP 19

Lessons from clinical studies

0109

The effects of long-term pioglitazone therapy on lipid profiles in high-risk type 2 diabetes patients: results from PROactive

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Background and Aims: Dyslipidemia associated with type 2 diabetes is characterized by elevated triglyceride (TG) levels and decreased high-density lipoprotein cholesterol (HDL-C) levels. Pioglitazone (PIO) has shown benefits in lipid metabolism in patients with type 2 diabetes, in addition to its glucose-lowering effects. Subanalyses of patients in PROactive have assessed the lipid-altering effects in four cohorts: PIO vs placebo (PBO), PIO vs PBO with or without insulin at baseline, PIO when added to metformin (MET) or sulfonylurea (SU) monotherapy, and PIO added to MET+SU combination therapy.
Materials and Methods: PROactive was a randomized, double-blind, placebo-controlled cardiovascular outcome study in which 5,238 patients received randomized treatment of either PIO or matching PBO, in addition to other blood-glucose and cardiovascular medications. Doses of PIO were titrated from 15 mg to 30 mg at the first month visit, and to 45 mg at the second month visit, where it was maintained unless there were tolerability concerns. Mean follow up was 34.5 months and mean exposure to study medication was 30 months.
Results: There were statistically significantly greater mean decreases in TG levels and mean increases in HDL-C levels in the PIO group compared with other treatments in all four cohorts of patients. Despite a significant increase in LDL-C with PIO, the LDL-C/HDL-C ratio was significantly improved compared to PBO for those receiving and not receiving insulin at baseline. Similar improvements in LDL-C/HDL-C ratios were seen between the PIO vs PIO+MET and/or SU treatment groups.

Changes from baseline in lipid parameters. PIO vs PBO and PIO with or without insulin at baseline

			Receiving any combination of insulin		Not receiving insulin	
Mean % change from baseline	PIO (N=2605)	PBO (N=2633)	PIO (N=864)	PBO (N=896)	PIO (N=1741)	PBO (N=1737)
TG (mmol/L)	−1.7	+12.5	−2.21	+14.11	−1.41	+11.7
HDL-C (mmol/L)	+21.2	+11.3	+20.5	+10.1	+21.6	+11.9
LDL-C (mmol/L)	+12.1	+8.4	+15.1	+12.1	+10.6	+6.5
LDL-C/HDL-C	−4.39	−0.04	−0.95	+4.66	−6.1	−2.5

Changes from baseline in lipid parameters: PIO+MET or SU and PIO+MET+SU

Mean % change from baseline	PIO+MET (N=253)	PBO+MET (N=261)	PIO+SU (N=508)	PBO+SU (N=493)	PIO+MET+SU (N=654)	PBO+MET+SU (N=660)
TG (mmol/L)	−2.60	+14.94	−1.09	+11.70	−1.1	+8.3
HDL-C (mmol/L)	+20.62	+10.37	+21.82	+12.67	+21.2	+12.6
LDL-C (mmol/L)	+12.60	+10.03	+9.83	+4.76	+12.3	+6.1
LDL-C/HDL-C	−2.67	+1.30	−6.86	−5.31	−4.4	−2.6

Conclusion: Improvements in lipid parameters were observed in the PIO group on top of the known benefits of the concomitant glucose-lowering and lipid-altering medications taken in all of the treatment groups.

0110

Effect of simvastatin and/or pioglitazone on adiponectin, proinsulin and glucose metabolism in non-diabetic patients at cardiovascular risk – the PIOSTAT Study

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Background and Aims: Insulin resistance and impaired postprandial glucose metabolism are well known confounders in cardiovascular disease. We investigated the effect of acute intervention with Pioglitazone in comparison to and in combination with Simvastatin on insulin resistance, plasma adiponectin levels, and the postprandial time course of plasma glucose, insulin and proinsulin concentrations in a non-diabetic cardiovascular risk population.

Materials and Methods: 125 non-diabetic patients with increased cardiovascular risk, defined as hypertension and/or a previously sustained cardiovascular event (47 male, 78 female; age 58.6±6.3 years; BMI 30.8±3.2 kg/m^2; mean±SD) were included in our three arm, double blinded, double dummy — parallel study. Patients were randomised to 45 mg Pioglitazone and Plazebo (PIO), 45 mg Pioglitazone and 20 mg Simvastatin (PIO/SIM), or Plazebo and 20 mg Simvastatin (SIM). At baseline and after 3 months, fasting blood samples were taken for the measurement of glucose, insulin and adiponectin concentrations and the HOMAs Score was calculated. In addition, an oral glucose load (OGTT) with the measurement of glucose, insulin and proinsulin intact at 0, 30 and 120 minutes was performed.

Results: The HOMAs Score declined from 3.27±2.21 to 2.40±0.97 ($p=0.003$) during PIO treatment and from 3.85±2.19 to 2.81±1.15 ($p=0.0003$) during treatment with PIO/SIM. Inversely, adiponectin plasma levels increased from 14.0±8.2 to 27.6±14.5 μg/mL ($p<0.0001$) during PIO and from 11.7±10.0 to 26.7±15.7 μg/mL ($p<0.0001$) during PIO/SIM treatment. A slight decrease in adiponectin levels from 15.5±12.7 to 11.6±7.0 μg/mL ($p<0.05$) could be observed during SIM treatment. An inverse correlation was found between the change in $HOMA_S$ Score and plasma adiponectin levels ($r=-0.31$, $p=0.0004$). In addition, treatment with PIO and PIO/SIM resulted in an overall improvement in glucose and insulin levels during OGTT ($p<0.05$, respectively). As shown in table 1, treatment with PIO and PIO/SIM significantly reduced postprandial glucose and proinsulin levels. Significant improvements in total cholesterol, HDL and LDL levels could be observed during PIO/SIM and SIM treatment ($p<0.05$ respectively), but not during single treatment with PIO.

Conclusion: Despite every single drug evolves beneficial effects on distinct cardiovascular risk parameters, only the combined treatment with both study drugs was sufficient to

provide an overall improvement in fasting and postprandial cardiovascular risk predictors in a non-diabetic population at cardiovascular risk.

Table 1: Glucose and Proinsulin Levels during OGTT (mean±SD; * p<0.05 vs baseline)

		0′	0′	30′	30′	120′	120′
		Baseline	12 weeks	baseline	12 weeks	baseline	12 weeks
Glucose (mmol/L)	PIO	5.6± 0.5	5.2± 0.5 *	9.0± 1.7	7.9± 1.8 *	5.9± 1.5	5.4± 1.1 *
Glucose (mmol/L)	SIM	5.6± 0.6	5.6± 0.6	9.3± 1.5	9.4± 1.6	6.0± 1.5	6.2± 1.6
Glucose (mmol/L)	PIO+ SIM	5.7± 0.7	5.5± 0.7 *	9.8± 1.8	8.3± 1.8 *	6.8±2.0	6.0±1.7 *
Proinsulin intact (pmol/L)	PIO	12.2± 15.6	11.6± 15.0	20.5± 18.1	16.7± 15.0 *	29.6± 21.4	22.1± 17.5 *
Proinsulin intact (pmol/L)	SIM	8.1± 7.4	8.1± 6.3	15.9± 11.4	16.5± 11.6	27.2± 19.5	28.2± 16.4
Proinsulin intact (pmol/L)	PIO+ SIM	8.8± 8.6	7.4± 5.8	16.7± 13.6	11.6± 8.9 *	24.3± 17.1	21.2± 16.5 *

Supported by TAKEDA

0111

Cardiovascular risk modification after combined intervention of rosiglitazone and exercise in patients with type 2 diabetes mellitus and metabolic syndrome

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Background and Aims: Growing evidence indicates that rosiglitazone (ROSI) and exercise confer beneficial effects on numerous cardiovascular (CV) risk factors. The aim of this study was to determine the CV effects of combined intervention of ROSI and exercise training program in patients with type 2 diabetes mellitus (NIDDM) and metabolic syndrome (MS).

Materials and Methods: A total of 100 individuals with NIDDM and MS, aged 44–70 years, without micro-/macrovascular complications entered this 6month study. All the participants were overweight (BMI>27 kgr/m^2), with inadequate glycemic control (HbA1c>7%) under stable anti-diabetic regimen with 1700 mgr metformin and 180 mgr gliclazide daily. At baseline and at the end of the study all the patients performed a graded exercise test and blood samples were drawn to determine biochemical parameters. The patients were randomly assigned to the following groups: A) (ROSI+EX, N=25) Patients received 4 mgr/d of ROSI as added-on therapy and performed a supervised EX program (4 times/week) for 6 months. B) (ROSI, N=25) ROSI (4 mgr/d) was administered additionally for 6 months. C) (EX, N=25) Diabetics participated only in EX program similar to group A. D) (Control, N=25) The rest of participants served as control group. We used one-way ANOVA and post-hoc Tukey test for the comparison between groups.

Results: At the end of the study we observed a significant reduction of HbA1c and fasting plasma glucose, respectively, in group: A (−1.67±0.87% and −43.58±27.02 mg/dl), B (−0.96±0.4% and −39.96±26.58 mg/dl), C (−0.51±0.21% and −20.15±19.14 mg/dl), while glycemic control deteriorated in group D (+0.34±0.06%, 10.32±3.63 mg/dl). With the exception of control group systolic/diastolic blood pressure was reduced considerably in group A (−13.08±11.28/−7.31±7.53 mmHg), group B (−7.32±7.64/−3.93±4.16 mmHg) and group C (−7.86±9.69/−2.9±4.33 mmHg). Exercise program contributed to significant reduction of cholesterol and LDL (p<0.05) in group A and C. The latter lipid parameters remained unaltered in groups B and D. In the following table the additive effects of ROSI and EX in HDL and hs-CRP are easily recognized. Concerning insulin resistance, triglycerides and VO2max the combined intervention revealed the synergistic effects of ROSI and EX in group A. Finally the performance of systemic exercise seemed to counteract the weight gain of ROSI treatment.

Change of variables	A	B	C	D
Weight (kgr)	0.09±0.18*	2.52±1.27*	−1.59±0.71*	0.61±0.24
hsCRP (mg/dl)	−0.29±0.031*	−0.17±0.02*	−0.15±0.01*	0.043±0.01
Insulin (mU/lt)	−5.33±3.21*	−2.7±1.58*	−2.16±1.1*#	1.25±0.33
HOMA	−4.06±1.91*#	−1.99±1.06*	−1.59±0.65 *	0.96±0.42
HDL (mg/dl)	11.08±4.87*#	3.86±2.04*	6.59±4.97 *	−2.61±1.38
VO2max (ml/kgr/min)	7,98±1,44*#	2,08±0,39*	4,32±2,14*	−1,26±0,75
Triglycerides (mg/dl)	−32.39±3.20*	−7.33±3.83	−19.43±3.63*	16.61±4.94

Conclusion: The combined therapy with ROSI and exercise resulted in multiple beneficial effects on CV risk profile in patients with NIDDM and MS. These findings indicate that ROSI in conjunction with physical activity exert a greater cardioprotective role in high risk patients.
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0112

An analysis from PROactive on the effects of pioglitazone on major adverse cardiovascular events (MACE) and myocardial infarction

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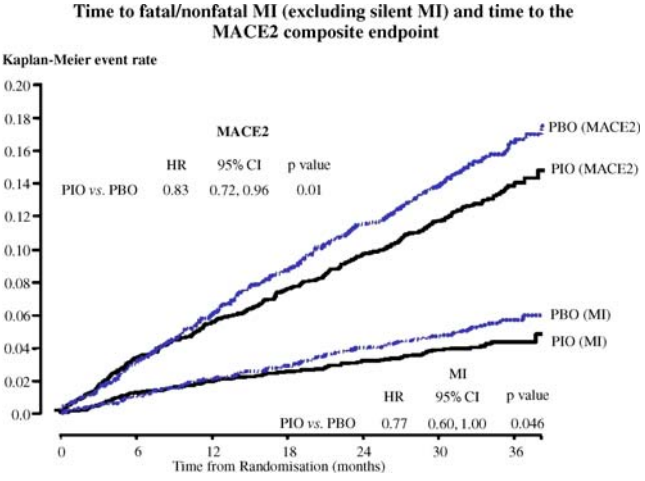
Background and Aims: Composite endpoints of cardiovascular events are commonly used to compare treatments in large cardiovascular outcome studies and are referred to as major adverse cardiovascular events (MACE). Patients with type 2 diabetes are at a higher risk of macrovascular events than the general population. In this analysis from PROactive, we evaluated the effects of pioglitazone on the prespecified endpoints of fatal/non-fatal myocardial infarction (MI; excluding silent MI) and a MACE1 composite of cardiovascular death, non-fatal MI (excluding silent MI), or non-fatal stroke; and on a *post-hoc* MACE2 composite of all-cause mortality, non-fatal MI (excluding silent MI), non-fatal stroke, or acute coronary syndrome in high-risk patients with type 2 diabetes.
Materials and Methods: PROactive was a double-blind, placebo-controlled cardiovascular outcome study in which 5,238 patients were randomised to pioglitazone or placebo, in addition to their existing glucose-lowering and cardiovascular medication. Patients started with 15 mg/day of pioglitazone, titrated to maximum tolerated doses in 15 mg increments to a maximum of 45 mg. Mean follow-up was 34.5 months.
Results: A Cox proportional hazards model showed statistically significant risk reductions with pioglitazone compared with placebo for the prespecified endpoints of MI (23%; HR=0.77; 95%CI=0.60,1.00; P=0.046) and MACE1 (18%, HR=0.82; 95% CI=0.70,0.97; P=0.020), and the post hoc MACE2 endpoint (17%; HR=0.83; 95%CI=0.72,0.96; P=0.010). At study end, 339 (13.0%) patients in the pioglitazone group had a first event that contributed to the MACE2 endpoint, compared with 409 (15.5%) in the placebo group and 108 (4.1%) pioglitazone patients and 140 (5.3%) placebo patients had an MI (excluding silent MI). A similar result occurred when non-cardiovascular deaths were excluded from the MACE2 endpoint (HR=0.80; 95%CI=0.69,0.94; P=0.005).



Conclusion: Pioglitazone treatment resulted in a 23% clinically relevant and statistically significant risk reduction in the prespecified time to first fatal/non-fatal MI (excluding silent MI) and nearly a 20% reduction in time to MACE1 compared with placebo in this population of patients with advanced type 2 diabetes at high risk for cardiovascular events.

0113

Long-term treatment with pioglitazone improves markers of liver function in patients with type 2 diabetes: results from PROactive

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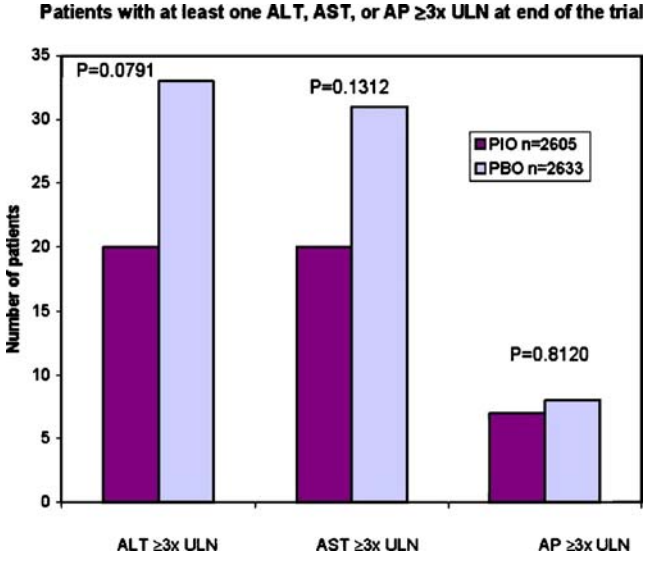
Background and Aims: Due to hepatotoxicity concerns, liver function monitoring is recommended in patients receiving thiazolidinedione treatment. Pioglitazone was administered to 5,238 high-risk patients with type 2 diabetes and macrovascular disease in PROactive, a cardiovascular outcome study. We examined the effects of pioglitazone on liver safety in this patient population.
Materials and Methods: PROactive was a double-blind, placebo-controlled study in 5,238 patients with type 2 diabetes

and macrovascular disease randomised to pioglitazone or matching placebo in addition to existing glucose-lowering and cardiovascular medication, including anti-hypertensive, anti-platelet, and lipid-modifying drugs. The starting dose of pioglitazone (15 mg) was titrated to a maximum tolerated dose of 45 mg. Mean follow-up was 34.5 months. Laboratory assessments for liver function (alanine aminotransferase [ALT], aspartate aminotransferase [AST], and alkaline phosphatase [AP]) were performed at every visit during the first year and every 6 months thereafter.

Results: The results show that there was a general shift toward normalisation of ALT and AST values in the pioglitazone group from baseline to the final visit compared to no change or an increase in values in the placebo group. At study entry, similar proportions of pioglitazone and placebo patients had ALT (9.7% and 10.7%), AST (6.1% and 6.9%), or AP (6.7% and 5.8%) values above the upper limit of normal (>ULN), respectively. In the pioglitazone group at final visit, the occurrence of elevated ALT decreased to 5.9%, whereas there was a small increase in the placebo group (11.9% at final visit; P<0.0001 between groups). More placebo than pioglitazone patients also had elevated levels of AST (7.3% *versus* 4.5%; P=0.0001), respectively. There was a small decrease in the proportion of patients with AP values>ULN in the pioglitazone group (to 5.9%) and a small increase in the placebo group (to 6.6%; P=NS). In the pioglitazone group, mean ALT decreased from 28.3 IU/L at baseline to 26.2 IU/L at the final visit, whereas there was a small mean increase (2.0 IU/L) in the placebo group (P<0.0001 between groups).



Conclusion: Results show that long-term pioglitazone treatment was associated with an improved hepatic laboratory profile compared with placebo in this high-risk patient population with type 2 diabetes. Because ALT is a marker of the amount of liver fat related to liver insulin resistance, these data suggest that treatment with pioglitazone is associated with a lowering of liver fat content.

0114

Evaluation of ezetimibe/simvastatin versus rosuvastatin in hypercholesterolaemic patients with type 2 diabetes or metabolic syndrome

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Background and Aims: Type 2 diabetes mellitus (DM) is considered a coronary heart disease (CHD) risk equivalent. DM and metabolic syndrome (MS) share elements of an atherogenic dyslipidemia characterized by high levels of triglycerides (TG), low levels of high-density lipoprotein cholesterol (HDL-C), and small, dense low-density lipoprotein (LDL) particles. This post-hoc analysis compared the efficacy of ezetimibe/simvastatin (E/S) vs. rosuvastatin (R) in patients with DM, MS without DM, or neither disorder.

Material and Methods: This was a multicenter, double-blind, randomized, parallel-group, 6-week study in which patients were randomized equally to 1 of 6 treatment arms: E/S 10/20, 10/40, or 10/80 mg, or R 10, R 20, or R 40 mg. In this post-hoc analysis, the efficacy of E/S was compared with R at the usual start doses, E/S 10/20 mg vs. R 10 mg; alternate start doses, E/S 10/40 vs. R 20; maximum doses, E/S 10/80 vs. R 40, and averaged across all doses, All E/S vs. All R.

Results: Of 2959 patients randomized, 2855 patients were included in the modified intent-to-treat population (E/S=1428, R=1427), of which 367 (12.9%) had DM, 811 (28.6%) had MS without DM, and 1661 (58.5%) had neither disorder. Sixteen patients, who did not have DM, could not be placed in a category because they were missing other data that precluded their MS classification. Efficacy results are shown below for LDL-C, HDL-C, and TG, averaged across all doses (All E/S vs. All R).

Parameter	DM		MS without DM		Neither	
Mg/dL	E/S (n=186)	R (n=181)	E/S (n=395)	R (n=416)	E/S (n=837)	R (n=824)
LDL-C						
Baseline†	170.0	169.8	174.2	172.9	173.0	173.8
% change‡	−58.5 (0.9)	−54.2 (0.9)	−55.0 (0.6)	−51.8 (0.6)	−55.6 (0.4)	−51.0 (0.4)
Tx Diff§**	−4.3 (−6.7, −1.9)		−3.2 (−4.8, −1.6)		−4.6 (−5.7, −3.4)	
HDL-C						
Baseline†	46.6	47.0	44.4	44.2	53.9	53.8
% change‡	7.0 (0.8)	6.3 (0.8)	9.4 (0.5)	8.9 (0.5)	6.9 (0.4)	7.3 (0.4)
Tx Diff§	0.7 (−1.4, 2.9)		0.5 (−0.9, 1.9)		−0.4 (−1.4, 0.6)	
TG						
Baseline††	172.8	185.0	210.0	203.8	129.5	132.5
% change††	−28.2	−26.7	−31.6	−29.2	−23.4	−21.5
Tx Diff‡‡**	−3.0 (−6.7, 0.7)		−2.7 (−5.2, −0.4)		−2.8 (−4.7, −0.8)	

†Means; ‡LS Means (SE); §LS Means (95% CI); ††Median; ‡‡Median (95% CI); **p<0.001 for treatment effect, treatment by subgroup interaction NS.

Conclusion: DM and MS patients treated with ezetimibe/simvastatin showed greater improvements in LDL-C and TG, with similar improvements in HDL-C, compared with those treated with rosuvastatin. Results were similar for the individual dose comparisons. These findings were consistent with the main study results. Thus, the dual cholesterol-lowering mechanisms of ezetimibe/simvastatin provided additional lipid-modifying benefits compared to rosuvastatin monotherapy in patients with DM and MS without DM.
Supported by Merck/Schering Plough Pharmaceuticals

OP 20

Glucose metabolism and the brain

0115

Prominent decrease of hippocampal neurogenesis in a spontaneous model of type 1 diabetes, the nonobese diabetic mice (NOD): a probable link with diabetes associated-depression?

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Background and Aims: The production of new neurons (neurogenesis) in the adult brain is restricted to two brain areas, the subventricular zone and the dentate gyrus (DG) of the hippocampus. In the DG, this plastic event is associated to specific types of learning and is especially modulated by hormones, environmental factors (stress, exercise) and aging. Defective neurogenesis is intimately linked to inflammation, affective-related disorders and depression. In human diabetes, degenerative and functional disorders of the central nervous system, including a high incidence of depression, are common findings. We recently reported that DG neurogenesis is reduced in streptozotocin (STZ)-treated mice, a pharmacological model of T1D. Here, we wanted to explore DG neurogenesis before and after diabetes onset in the NOD mouse, a well described spontaneous T1D model.
Materials and Methods: DG cell proliferation and survival were studied as a function of age in prediabetic and diabetic NOD and control C57BL/6 and BALB/C female mice, using different protocols of 5-bromo-2′-deoxyuridine (BrdU) incorporation, and cell phenotype analysis by immunocytochemical co-detection for glial (GFAP) or neuronal (Neu-N) markers and confocal microscopy.
Results: In the first protocol, cell proliferation was assessed in the DG of 4, 8 and 12-week-old control strains and NOD mice (the 12-week-old NOD mice were overtly diabetic, whereas the 4- and 8-week-old groups were pre-diabetic). Levels of BrdU positive cells in NOD DG were, regardless of age, constantly and significantly lower than in controls, showing that hippocampal alterations are present far before diabetes onset in NOD mice. The second protocol was designed to study survival of the newly-generated cells: 15-week-old animals were sacrificed 21 days after BrdU injection. Here, in addition to controls, two subsets of NOD mice were considered: non diabetic (noDb) and diabetic (Db). Cell survival in NOD DG was significantly lower than C57BL/6, where 599.4±78.9 BrdU+cells were found per DG while the values corresponding to NOD Db were 138.9±27.2 ($p<0.001$) and NOD noDb, 313.6±41.0 ($p<0.01$). Moreover, the presence of diabetes strongly aggravated cell survival in DG ($p<0.05$). However, confocal analysis of cell phenotypes determined that the proportion of neurons in these groups was not altered by the diabetic state.
Conclusion: Like in the pharmacological (STZ) T1D model, hippocampal neurogenesis is strongly diminished in the spontaneous T1D model of the NOD mouse. Notably, in the STZ model, we previously showed that antidepressant treatment (fluoxetine) induced complete recovering of proliferation of cells potentially becoming neurons, an effect that might result from improvement of hypothalamo-pituitary-adrenal axis that is known to be altered in rodent and human diabetes. Here, in addition, we showed that hippocampal DG cell proliferation defect takes place during the prediabetic stage in the NOD mouse. Whether this early alteration might result, in this autoim-

mune strain, from HPA axis alterations and/or ongoing brain inflammatory process remains to be investigated.
Supported by Inserm-Conicet International Agreement, University of Buenos Aires Grant.

0116

Hippocampal neuronal death and gliosis during development of streptozotocin diabetes mellitus

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Background and Aims: Diabetes mellitus is characterized by hypothalamo-pituitary-adrenal (HPA) axis hyperactivity, shown both in experimental and clinical studies. However, mechanisms of this phenomenon remains still unclear. It is suggested that diabetes-induced hippocampal damage can produce morphological substrate for diminished glucocordicoid negative feedback regulation. The present investigation was objected to study expression of pro- and anti-apoptotic factors and glial reaction in hippocampus at different terms of experimental diabetes.
Materials and Methods: Diabetes was induced by a single intraperitoneal streptozotocin administration (45 mg/kg) in adult female Wistar rats during diestrus. Animals were killed 3, 7, 14, 21 and 35 days after. Hippocampal expression of bcl2, inducible nitric oxide synthase (iNOS), neuronal enolase (Neu, specific neuronal marker) GFAP (astroglial marker) and BrdU were studied using indirect immunofluorescence. Additionally, blood corticosterone, insulin and glucose level were measured using ELISA and standard biochemical techniques.
Results: Diabetes development in all terms was characterized by progressive hypoinsulinemia and hyperglycemia; the blood costicosterone concentration was maximally increased at day 14. Starting from day 21 diabetes led to progressive hippocampal neuronal loss at all areas and at day 35 the maximum decrease (-43,0±5,2%) in number of neuronal cells was observed in CA3 area. This was associated with prominent astrogliosis: both number of astroglial cells in GFAP expression level was increased already at day 14 and later gliosis was further enhanced till the end of the experiments (35 days). iNOS expression was found in hippocampus prior to neuronal loss already at day 3 totally disappearing after the first week of diabetes development.
Conclusion: Thus, it can be concluded, that diabetes development is associated with progressive neuronal degeneration and gliosis in hippocampus. This process is tightly linked to the elevated blood corticosterone levels.
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0117

Early vascular risk factors impair brain metabolism in healthy men

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Background and Aims: Silent brain infarctions and cognitive decline are common findings in type 2 diabetes (T2D). Typical location for a silent brain infarction is the thalamus with its long penetrating end arteries. It is an important relay station for many neural loops that regulate the initiation and termination of planning, working memory, and attention. We hypothesized that early vascular risk factors may derange thalamic metabolism, which predispose brain tissue to later damage.
Materials and Methods: Eighteen healthy non-smoking men (22–45 yrs) without any medication and with normal brain MRI were studied. Nine subjects had a first degree relative with T2D and 3–4 of the following vascular risk factors: waist≥94 cm, fS-Gluc≥5.6 mmol/l, BP≥130/85 mmHg, S-Tg≥1.7 mmol/l, and S-HDL<1.03 mmol/l. The controls had no family history of T2D and 0–1 vascular risk factors. The groups were matched for age but differed (p<0.01) in weight (94±13 vs. 77±10 kg), waist (104±5 vs. 78±8 cm), systolic BP (141±11 vs. 126±8 mmHg), diastolic BP (88±4 vs.76±8 mmHg), and fS-Gluc (5.6±0.7 vs. 4.7±0.3 mmol/l). There was no difference in S-Chol (4.8±0.9 vs. 4.4±1.0 mmol/l), S-HDL (1.4±0.2 vs. 1.4±0.3 mmol/l), S-LDL (2.6±0.7 vs. 2.5±1.0 mmol/l) or S-Tg (1.7±1.2 vs. 0.9±0.3 mmol/l) between the groups. The risk-factor group underwent a 75-g OGTT: 2 h S-Gluc was 6.7±1.7 mmol/l.
Single-voxel short echo time (TE 30 ms) 1D-spectra were collected after 12 hours fast in the thalamus. Brain metabolites creatine (Cr; energy metabolite), N-acetyl aspartate (NAA; a neuronal marker), choline and myo-inositol (Cho and m-Ins; membrane markers), glucose (Gluc), glutamine and glutamate (Glx; neurotransmitters), and tissue water (H_2O) were evaluated by integration of the characteristic spectral regions. ^{1}H MRS studies were performed with a clinical 1.5 T MR-system using the PRESS method. Metabolites were referred to the unsuppressed tissue water signal.
Results: In the risk-factor group, Cr/H_20 was 14% higher in the thalamus (Table). Intensity of H_2O signal was similar in the groups (2.5±0.3 vs. 2.6±0.1·10^8 a.u.).

$\cdot 10^{-4}$	Cr/H_20	NAA/H_20	Cho/H_20	m-Ins/H_20	Gluc/H_20	Glx/H_20
Subjects	2.4±0.3	4.4±0.3	1.9±0.2	1.6±0.3	0.3±0.1	1.6±0.2
Controls	2.1±0.2	4.2±0.2	1.8±0.2	1.5±0.3	0.3±0.1	1.5±0.1
p	0.019	0.058	*NS*	*NS*	*NS*	*NS*

Cr levels correlated positively with OGTT 2 h S-Gluc concentration (r=0.75, p<0.05)

Conclusions: This data suggests that the Cr level in thalamus is increased in healthy men with early vascular risk factors. Increased Cr may indicate enhanced glial metabolism, activated possibly by alterations in glucose availability to neurons.

0118

Diabetes status and fasting plasma glucose are independent determinants of cognitive status in people at high risk for cardiovascular disease

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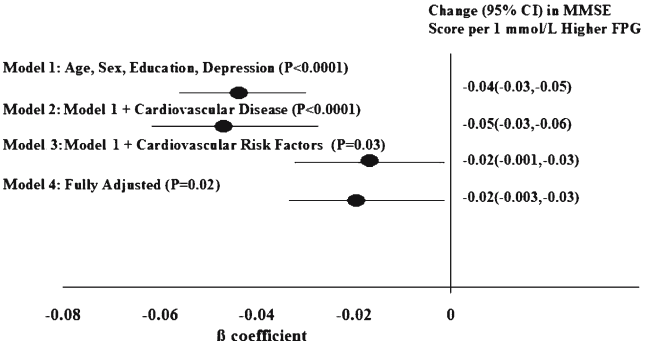
Background and Aims: Dementia and cognitive dysfunction (predementia) are a growing problem worldwide. Recent evidence suggests that elevated levels of glucose are an independent determinant of cognitive dysfunction. The objective of this study was to assess the cross-sectional relationship between measures of glycemia and cognitive status.
Materials and Methods: The ONgoing Telmisartan Alone and in combination with Ramipril Global Endpoint Trial (ON TARGET) (N=25,620) trial and the concurrent Telmisartan Randomized Assessment Study in ACE intolerant Subjects with Cardiovascular Disease (TRANSCEND) (N=5926) trials are two long term parallel international multi-centre randomized trials assessing the cardiovascular effects of an angiotensin receptor blocker (ARB), telmisartan and an angiotensin converting enzyme (ACE) inhibitor, ramipril in high risk individuals. Baseline data from both trials provide a unique opportunity to analyze the cross-sectional relationship between measures of glycemia and cognitive status. The Mini Mental State Examination (MMSE) was used to measure cognitive function. The relationship between diabetes status, fasting plasma glucose (FPG) and MMSE score was assessed; a B coefficient as well as a p value was reported. The p value indicates the statistical significance. The beta coefficient for diabetes status is the mean difference in MMSE score between those with and without diabetes. For FPG the B coefficient describes the mean difference in MMSE score for every 1 mmol/L difference in FPG value.
Results: In people with diabetes the mean MMSE score was 0.4 units lower than in people without diabetes (P<0.0001). In all participants, a 1 mmol/L higher FPG value was associated with a MMSE score that was 0.06 units lower (P<0.0001). The association persisted even after adjustment for several cardiovascular risk factors.
Conclusion: Dysglycemia is an independent determinant of cognitive function in people at high risk for cardiovascular events. Prospective studies of this relationship can discern the temporal relationship between these variables and clinical trials may reveal whether or not glucose lowering can affect the progression of cognitive decline in this population.

The association between FPG levels and MMSE score after adjusting for baseline characteristics



0119

Brainstem auditory evoked potentials in patients with diabetes mellitus

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Background and Aims: The evaluation of brainstem auditory evoked potentials provides the possibility to detect subclinical cerebral damage. Therefore, the aim of our study was to investigate the parameters of short-latency brainstem auditory evoked potentials in patients with type 1 and type 2 diabetes mellitus without clinical signs of cerebral or cochlear damage.
Materials and Methods: One hundred and five patients with diabetes mellitus were examined including 43 patients with type 1 and 62 with type 2 diabetes. Patients were divided into three age groups. The first group consisted of patients 17–39 years old (30 persons), mean age 26.1+1.3 years, BMI − 23.6+0.6 kg/m^2, HbAlc − 8.9+0.3%. The second group

included middle age patients − 40–59 years old (44 persons), mean age 50.2+0.8 years, BMI − 29.5+0.7 kg/m^2 , HbAlc − 8.8+0.3%. The third group consisted of elderly patients − 60–79 years (31 persons), mean age 65.7+0.7 years, BMI − 29.8+0.7 kg/m^2, HbAlc − 9.0+0.3%. Control group included 32 persons without history of diabetes mellitus who were also divided into three respective age-matched groups. Statistical analysis was performed by Student's test.

Results: We found the significant prolongation of the V wave latency time of auditory brainstem evoked potentials and the I–V interwave interval in diabetic patients in all age groups compared to age-matched controls. The latency of V wave in the first age group patients with diabetes was 5.71+0.04 ms compared to 5.58+0.02 ms in the control group; in the second age group − 5.79+0.02 ms and 5.61+0.02 ms; in the third age group − 5.86+0.03 ms and 5.62+0.03 ms, respectively, $p<0.05$ for all comparisons. Interwave interval I–V in the first age group of diabetic patients was 4.10+0.02 ms and 3.88+0.03 ms in the control group; in the second age group − 4.10+0.02 ms and 3.93+0.03 ms; in the third age group − 4.19+0.08 ms and 3.95+0.02 ms, in diabetic and control subjects, respectively, $p<0.05$.

Conclusion: The revealed prolongation of latency time of auditory brainstem evoked potentials could reflect the subclinical cerebral and cochlear impairments in patients with type 1 and type 2 diabetes mellitus.

0120

Pioglitazone in patients with or without a history of stroke: results from PROactive

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Background and Aims: The risk of stroke is markedly higher in patients with diabetes than in those without. The most effective strategies to prevent stroke among people with diabetes include antihypertensive, antiplatelet, and lipid-altering therapy; however, benefit has not been demonstrated with any glucose-lowering drug. No data have been published on the potential benefit of the use of thiazolidinediones in preventing primary and secondary stroke (all stroke) in patients with type 2 diabetes. We conducted a prespecified analysis from PROactive of the risk of stroke and other cardiovascular outcomes in patients with type 2 diabetes with and without prior stroke (>6 months before randomisation).

Materials and Methods: The prospective, double-blind PROactive trial randomised 5,238 patients with type 2 diabetes and increased risk of cardiovascular disease to pioglitazone (force-titrated to 45 mg) or placebo, in addition to current medication for diabetes and cardiovascular disease. Cardiovascular endpoint events were independently adjudicated.

Results: In the patients with previous stroke, there were statistically significant benefits of pioglitazone on the endpoints of 1. first fatal or non-fatal stroke and 2. cardiovascular death, MI (excl. silent MI), or stroke. There was a trend of benefit with pioglitazone for the primary and the principal secondary endpoints. Thirteen patients in the pioglitazone group (2.7%) and 16 in the placebo group (3.2%) had a transient ischaemic attack. There was no effect of pioglitazone on subsequent strokes in the patients without prior stroke.

Endpoints	Prior Stroke n events (%)			No Prior Stroke n events (%)		
	PIO N=486	PBO N=498	HR (95%CI)	PIO N=2119	PBO N=2135	HR (95% CI)
All-stroke	27 (5.6)	51 (10.2)	0.53 (0.34, 0.85)	59 (2.8)	56 (2.6)	1.06 (0.73, 1.52)
Cardiovascular death, MI or stroke[1]	63 (13.0)	88 (17.7)	0.72 (0.52, 1.00)	194 (9.2)	225 (10.5)	0.86 (0.71, 1.04)
Primary[2]	98 (20.2)	126 (25.3)	0.78 (0.60, 1.02)	416 (19.6)	446 (20.09)	0.94 (0.82, 1.07)
Principal secondary[3]	76 (15.6)	98 (19.7)	0.78 (0.58, 1.06)	225 (10.6)	260 (12.2)	0.86 (0.72, 1.03)
All-cause death	46 (9.5)	49 (9.8)	0.96 (0.64, 1.44)	131 (6.2)	137 (6.4)	0.96 (0.75, 1.22)

1. Excluding silent MI.
2. All-cause death, non-fatal MI (incl. silent MI), acute coronary syndrome, cardiac intervention including CABG or PCI, stroke, major leg amputation, or bypass surgery or revascularisation in the leg.
3. All-cause death, non-fatal MI (excl. silent MI), or stroke

Conclusion: PROactive was a large outcome study that examined the effects of pioglitazone on macrovascular outcomes and included stroke prevention in its composite endpoint. In a pre-specified subgroup analysis from this large-scale, prospective study, pioglitazone appeared to significantly reduce the risk of recurrent stroke in high-risk patients with type 2 diabetes and previous stroke. The addition of pioglitazone to other standard therapies used in stroke prevention appears to improve outcomes. This may be due to pioglitazone's other non-hypoglycemic effects such as its action on diabetic dyslipidemia and pleiotropic mediators.

OP 21

Inflammatory mediators in type 2 diabetes

0121

Sub-clinical inflammation in T2DM: the role of Toll like receptors in the activation of NFκB and cJun Kinase in the innate immune pathway in human abdominal isolated adipocytes and explants

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Background and Aims: Type 2 diabetes (T2DM) may result from a combination of obesity mediated hyperinsulinaemia/insulin resistance (IR) and sub-clinical inflammation. Our hypothesis is that hyperinsulinaemia/IR enhances absorption of endotoxin, which may have an effect on adipose tissue to elicit an inflammatory response. Our studies have shown circulating lipopolysaccharide (LPS), is significantly higher in T2DM than in BMI and aged matched controls ($p<0.001$, n=25), and is reduced by treatment with the insulin senstizer, rosiglitazone, in T2DM patients ($p<0.05$). In addition, toll-like receptors (TLR) 2 and 4, which are LPS receptors, are noted to increase with obesity and T2DM in human adipose tissue (AT). Therefore we investigated (1) the role of LPS in mediating the innate immune pathway in human AT (2) the resulting effect on adipocytokines release (3) the effect of blockade of inflammatory signaling molecules nuclear factor kappa B (NFκB, blocking agent SN50) and c-jun kinase (JNK) inhibitor (SP600125) on adipocytokine release (4) and the influence of explants versus isolated adipocytes in their inflammatory response.

Methods and Materials: Abdominal subcutaneous (AbdSc) adipose tissue explants and isolated AbdSc adipocytes were obtained from patients undergoing elective liposuction surgery (age 35.5±10.2 yrs; BMI 27.2±6.1 kg/m2; n=18). AbdSc adipocytes and explants were treated with and without LPS (10 ng/mL) and with or without NFκB or JNK blocker (n=8). Following treatment conditioned media was analysed for adiponectin, IL-6 and TNF-α. Protein expression of TLR 2 and 4 was also assessed (n=10).

Results: TLR-2 protein expression increased two-fold ($p<0.05$) compared with control in response to LPS treatment. TLR-4 protein expression remained unchanged. LPS treated AbdSc isolated adipocytes and explants both showed a significant increase in TNF-α and IL-6 response (IL-6, Control: 2700.0±40 pg/mL vs LPS: 4800±30 pg/mL; $p<0.001$; TNF-α, Control: 1.0±0.83 pg/mL vs LPS: 32.8±6.23 pg/mL; $p<0.0001$) and explants (IL-6, Control: 3300±90 pg/mL vs LPS: 1030±130 pg/mL; $p<0.001$; TNF-α, Control: 1.4±0.3 pg/mL vs 68.7±23.9 pg/mL; $p<0.05$). Adiponectin levels were unchanged in Abd Sc adipocytes but significantly reduced in explants ($p<0.05$). NFκB blockade reduced the levels of IL-6 in Abd Sc adipocytes (Control: 2.7±0.5 ng/mL vs Blocker: 2.1±0.4 ng/mL; $p<0.001$) whilst there was no significant change in TNF-α or adiponectin levels. In AbdSc explants however there were significant reductions in both TNF-α and adiponectin (TNF-α, Control: 1.4±0.3 pg/mL vs Blocker: 1.2±0.2 pg/mL; $p<0.05$: Adiponectin, Control: 0.47±0.07 μg/mL vs Blocker: 0.32±0.05 μg/mL; $p<0.01$) with a trend towards reduction in IL-6 (3.3±0.9 ng/mL vs 2.4±0.7 ng/mL; p=0.069). JNK blockade appeared to have more effect on adipocytokines reduction in explants than Abd Sc adipocytes.

Conclusions: In summary our studies indicate that LPS increases TLR 2 in isolated adipocytes, elicites an inflammatory response and reduces adiponectin secretion and that blockade of NFκB and or JNK pathways reduces cytokine release in human AT. These findings may be important for targeting new therapies to reduce inflammation. Thus LPS action in AT may represent a factor in initiating inflammation and therefore insulin resistance, and represent an important mechanism for pathogenesis of type 2 diabetes.

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0122

An LPS/CD14 mechanism is responsible for the onset of high-fat diet-induced obesity and insulin resistance

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Background and Aims: A recent ongoing mechanistic hypothesis for high-fat diet (HFD) induced-insulin resistance and obesity is that inflammation would trigger cytokine release impairing energy homeostasis and adipose tissue plasticity. However, no HFD-linked molecular mechanism has been proposed for the triggering of inflammation. Among natural inflammatory reagents, the bacterial endotoxin (LPS) is a potent candidate because it triggers inflammatory cytokines secretion by macrophages upon binding to its membrane receptor, it is ubiquitously distributed in the environment, and it is transported in the blood by triglyceride-enriched lipoproteins.

Materials, Methods and Results: To demonstrate this hypothesis we show that a fat enriched-diet increases fasted endotoxemia in healthy by 100% humans and by 40% in mice. To determine how elevated endotoxemia contributes to the development of obesity and diabetes, we challenged CD14 knockout mice with a HFD. Such mice were protected against LPS-induced cytokine secretion (IL6, IL1b, PAI-1). After 24 weeks of HFD, the degree of HFD-induced obesity and liver steatosis was reduced by 40%. To avoid the confounding effect of excessive body weight on insulin resistance we studied insulin sensitivity by an euglycaemic clamp after only 5 weeks of HFD, when diabetes is present but obesity undetectable. Under these conditions, insulin resistance was blunted in CD14 mutant mice. Furthermore, fasted hyperglycemia and glucose intolerance were totally prevented. These observations are likely due to tissue rather than circulating cell types, as mice transplanted with CD14-depleted hematopoietic cells retained LPS-induced insulin resistance.

Conclusion: Our data demonstrate that LPS is a potent inflammatory factor that during high-fat feeding triggers increased cytokine secretion, obesity and insulin resistance. Strategies aimed at lowering LPS in food and/or plasma may delay the development of metabolic diseases and obesity in humans.

0123

Monocyte chemoattractant protein-1 in obesity and type 2 diabetes. Relationship with insulin-resistance and proinflammatory cytokines

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Background and Aims: Monocyte chemoattractant protein (MCP-1) mRNA is elevated in the adipose tissue of genetically obese mice remaining responsive to exogenous insulin both in mice and in insulin-resistant 3T3L-1 adipocytes. It has been shown that MCP-1 may be released from human preadipocytes as well as from isolated mature adipocytes, with an increased secretion and expression in human obesity. In humans, the correlation between circulating MCP-1 (cMCP-1) and its local adipose tissue secretion has shown discordant results.

We studied the relationship between cMCP-1 plasma levels and insulin-resistance in a large group of healthy Caucasian male to whom S_i test was performed. Additionally, we aimed to investigate MCP-1 adipose tissue gene expression in relation with cMCP-1 plasma protein levels in a representative group of non-diabetic and Type 2 diabetic subjects with different degrees of obesity

Materials and Methods: cMCP-1 were measured in n=116 consecutive healthy male subjects to whom insulin sensitivity (S_i) test was performed. Circulating levels of sCD14, sTNFR2, sIL-6 and adiponectin were also measured in this population. Subcutaneous adipose tissue samples were obtained from n=107 non-diabetic and Type 2 diabetic subjects with different degrees of obesity. Real Time PCR was used to measure gene expression of *MCP-1*, *CD68, TNF*-α and its receptor *TNFR2*. cMCP-1 plasma levels were also measured.

Results: In the S_i study no independent effect of cMCP-1 levels on insulin sensitivity was observed. In the expression study, in non-diabetic subjects, MCP-1 mRNA had a positive correlation with BMI (r=0.407, p=0.003), TNF-α mRNA (r=0.419, p=0.002) and TNFR2 mRNA (r=0.410, p=0.003). In these subjects cMCP-1 were found to correlate with WHR (r=0.322, p=0.048). In patients with Type 2 diabetes, MCP-1 mRNA was up regulated compared to non-diabetic subjects. TNF-α mRNA was found to independently contribute to MCP-1 mRNA expression. In this group, CD68 mRNA was found to correlate with BMI (r=0.455, p=0.001).

Conclusions: cMCP-1 is not associated with insulin sensitivity in apparently healthy men. TNF-α is the inflammatory cytokine associated to MCP-1 expression in subcutaneous adipose tissue of non-diabetic and Type 2 diabetic subjects, suggesting a local inflammatory role of MCP-1 in this tissue with little or very weak contribution into circulating levels.

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0124

The *in vitro* effects of JNK of NF-κB inhibitor on resistin secretion from isolated human abdominal subcutaneous adipocytes

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Background and Aims: Obesity-associated low-grade inflammation is one of the foremost contributory factor in

the pathogenesis of type 2 diabetes mellitus (T2DM). Resistin has recently been implicated as a pro-inflammatory cytokine in obesity-related type 2 diabetes. The aims of this study were to characterise a role for resistin in sub-clinical inflammation, specifically the innate immune signaling pathway in human abdominal subcutaneous (AbdSc) adipose tissue (AT). Therefore, for this study we investigated (1) whether resistin could act as a positive mediator of cytokine production in isolated abdominal subcutaneous (Abd Sc) adipocytes; (2) the acute in-vitro effects of human recombinant (h–r) resistin on the expression of components of the innate immune pathway, specifically the nuclear factor-kappa B (NF-κB) and c-Jun N-terminal kinase (JNK) signaling pathways; (3) determine whether a regulatory feedback mechanism exists to reduce resistin secretion through use of JNK and NF-κB inhibitors, in isolated adipocytes and matched Abd Sc explants.

Materials and Methods: Isolated human Abd Sc adipocytes were treated with h–r resistin (10–50 ng/mL), NF-κB inhibitor (50 μg/mL) or JNK inhibitor (10 μM/mL). The response of components of the innate immune pathway to h–r resistin was determined by Western blot. Additionally, the level of resistin secretion from isolated adipocytes and IL-6 and TNF-α was examined by ELISA.

Results: Both TNF-α and IL-6 secretion were increased in response to h–r resistin (TNF-α, $p<0.001$; IL-6, $p<0.001$). Our findings also demonstrated that h–r resistin upregulated components of the NF-κB pathway. Protein expression of MyD88 (activated upon Toll-like receptor stimulation; n=4, $p<0.01$) and NF-κB (n=4, $p<0.05$) were increased in response to h–r resistin. Furthermore, when examining the IKK complex, whilst there was no effect on IKK-γ, IKK-β was significantly upregulated by h–r resistin treatment (n=6, $p<0.01$). Similarly, h–r resistin significantly upregulated mediators of the insulin signaling pathway, in particular, phosphospecific JNK-1 and JNK-2 protein expression was increased in response to h–r resistin treatment (JNK-1: n=6, $p<0.05$; JNK-2: n=6, $p<0.001$). Finally, AbdSc adipocytes treated with either NF-κB or JNK inhibitor significantly reduced the level of resistin secretion (NF-κB: Control, 124.4±41.1 pg/mL; NF-κB inhibitor, 85.5±24.8 pg/mL; n=8, $p<0.01$; JNK: Control, 101.5±19.2 pg/mL; JNK inhibitor, 77.0±11.4 pg/mL; n=6, $p<0.01$). However, whilst AT explants treated with NF-κB blocker reduced resistin secretion significantly (Control, 1222.9±195.4 pg/mL; NF-κB inhibitor, 913.0±147.4 pg/mL; n=8, $p<0.05$), no significant effect on resistin secretion from adipocytes was observed when inhibiting JNK (n=6).

Conclusions: These findings suggest resistin could participate in more than one mechanism for the upregulation of pro-inflammatory cytokine release from isolated adipocytes, potentially via the integration of NF-κB and JNK signaling pathways. Furthermore, this study emphasises potential crosstalk between insulin signaling and inflammatory pathways. Collectively, this study emphasises potential crosstalk between insulin signaling and inflammatory pathways with these metabolic functions being dysregulated in obesity and type 2 diabetes.

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0125

$Tace^{+/-}$ mice are protected from obesity and type 2 diabetes

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Background and Aims: Soluble TNF-alpha is believed to contribute to insulin resistance in prediabetes and type 2 diabetes. We reported that an increased activity of TNF-alpha Converting Enzyme (TACE) increases soluble TNF-alpha levels in Insulin Receptor null mice shifting the phenotype from normal glucose homeostasis to hyperinsulinemia/hyperglycemia. Moreover, increased Tace activity has been recently linked to development of atherosclerosis and chronic kidney disease in murine models.

Methods and Materials: To gain insights the role of TACE in insulin resistance related diseases we fed Tace+/− and WT littermates with a High-Fat Diet (HFD) for 16 weeks, studying glucose and lipid metabolism at the beginning and at the end of the study for each genotype.

Results: Growth curves showed that Tace+/− mice gained less weight compared with WT littermates ($p<0.01$, ANOVA–2). Interestingly, WT mice tend to develop hyperinsulinemia and have higher glucose levels compared to Tace+/− mice ($p<0.01$, ANOVA–1). IPGTT and ITT confirmed the protective effect of Tace haploinsufficiency against diet-induced insulin resistance. Wild-type mice showed abnormal glucose and insulin tolerance compared with $Tace^{+/-}$ mice ($p<0.01$, ANOVA-2). HFD WT mice showed increased fat pads compared with HFD $Tace^{+/-}$ mice ($p<0.01$, ANOVA-1). In HFD WT mice we observed as expected that the increase in WAT weight was associated with increased adipocyte cell size and decreased cell density. By contrast, in $Tace^{+/-}$ mice HFD did not cause a significant change in both cell density and size compared with mice fed a normal chow. Next, we inquired whether the protective phenotype determined by Tace inhibition under a HFD is explained by reduction of food intake or improvement in peripheral fat oxidation or both. Food intake was slightly but not significantly reduced in HFD $Tace^{+/-}$ mice compared with WT littermates. NEFA did not

increase and Adiponectin did not decrease in HFD Tace$^{+/-}$ compared to HFD WT mice. mRNA analysis revealed that Tace+/− compared with WT mice express significantly higher levels of Adiponectin and PPARgamma and lower levels of ccl-2/MCP-1 in WAT and increased UCP-1 expression in the skeletal muscle. Consistently, in db/db mice treatment with a Tace inhibitor, TAPI-1, with daily administration for 1 week led to improvement of fed glucose and insulin levels, total cholesterol and triglycerides levels ($p<0.05$ for each value compared to basal value, t-Test).

Conclusions: TACE inhibition in animal models, through genetic or pharmacological approach, significantly reduced the detrimental effect of overnutrition and obesity, regulating adipose tissue remodeling and gene expression in both WAT and muscle. Altogether these data favor the hypothesis that TACE inhibition may play a role in protecting from obesity, insulin resistance and dyslipidemia.

0126

Role of oxidative stress and inflammation in lipid-induced β-cell dysfunction

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Background and Aims: An important mechanism in pathogenesis of type 2 diabetes is elevation of plasma free fatty acids (FFA) which induce insulin resistance and may impair β-cell function and mass. In our previous studies the antioxidant taurine (TAU) prevented the impairment in β-cell function induced by prolonged exposure to FFA. TAU is a sulfur containing amino acid which may have other effects in addition to its antioxidant action. In support to taurine's action as an antioxidant, another antioxidant, N-Acetylcysteine (NAC), proved to be equally effective to TAU in preventing the FFA-induced impairment in glucose stimulated insulin secretion (GSIS); however, since NAC can be converted to TAU, it cannot be excluded that also the effect of NAC is independent of its antioxidant properties. Our current objective was to investigate the role of oxidative stress in the FFA-induced impairment in β-cell function using an antioxidant unrelated to TAU or NAC, TEMPOL (TPO, SOD mimetic), and to address potential downstream effects of oxidative stress involving inflammation.

Materials and Methods: Oleate (1.3 $\mu mol \cdot min^{-1}$) was infused iv for 48 h with or without the antioxidant TPO (2.41 $\mu mol \cdot kg^{-1} min^{-1}$) to elevate plasma FFA levels 2 fold in Wistar rats. After the 48 h infusion islets were isolated to evaluate GSIS ex vivo and to measure reactive oxygen species (ROS) by the dichlorofluorescein method. The same studies of 48 h oleate infusion or 48 h olive oil+ heparin infusion were also performed with or without the anti-inflammatory salicylate.

Results: GSIS in isolated islets was impaired by oleate (OLE: 1.009±0.102 pmol/islet/h, n=13, $p<0.01$ vs control: 1.618±0.224 pmol/islet/h, n=12, at 22 mM) but was restored by TPO (OLE+TPO: 1.421±0.200 pmol/islet/h at 22 mM, n=7, p=NS vs control) while TPO alone had no effect. Furthermore TPO abolished (p=NS OLE+TPO vs control) the increase in ROS induced by OLE in islets ($p<0.01$). Our laboratory has shown that the increase in ROS induced by lipids activates the IKKβ/NFkB inflammatory pathway in liver, inducing hepatic insulin resistance, which led us to our next objective, to determine whether IKKβ is also involved in lipid-induced decrease in GSIS. Oleate or an emulsion of 20% olive oil+heparin (40 $U \cdot ml^{-1}$) was infused as mentioned above, for 48 h in Wistar rats±the IKKβ inhibitor salicylate (0.117 $mg \cdot kg^{-1} min^{-1}$, the dose that inhibited IKKβ and prevented lipid-induced insulin resistance). After 48 h infusion plasma FFA were elevated 2 fold with either oleate or olive oil. GSIS in isolated islets was impaired by olive oil or oleate (olive oil: 0.996±0.132 pmol/islet/h at 22 mM, n=12; oleate: 1.033±0.097 pmol/islet/h at 22 mM, n=14, both $p<0.01$ vs. control: 1.542±0.159 pmol/islet/h at 22 mM, n=12) but was restored by salicylate (olive oil+ salicylate: 1.754±0.367 pmol/islet/h at 22 mM, n=6; oleate+ salicylate: 1.737±0.311 pmol/islet/h at 22 mM, n=8, both p=NS vs control) while salicylate alone had no effect (salicylate: 1.524±0.218 pmol/islet/h at 22 mM, n=10, p=NS vs. control).

Conclusion: Our results suggest a potential role for both oxidative stress and activation of inflammatory pathways involving IKKβ in lipid-induced β-cell dysfunction.

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OP 22

Mechanisms of prevention of endothelial damage

0127

Activation of 5′AMP-activated protein kinase induces NO-dependent vasodilation and uncovers insulin-mediated vasodilatation in muscle resistance arteries

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Background and Aims: Exercise enhances blood flow and insulin-mediated glucose uptake in muscle, but the underlying mechanisms are unknown. 5′-AMP-activated protein kinase (AMPK), which regulates whole-body insulin sensitivity, has been proposed to mediate these effects. Interaction of AMPK with insulin-mediated vasoreactivity in muscle may increase blood flow as well as insulin-mediated glucose uptake in muscle. Aim: To study the effects of AMPK activation on vascular tone, insulin-mediated vasoreactivity and insulin-mediated cell signaling in skeletal muscle resistance arteries.
Methods/Results: To study effects of AMPK activation, resistance arteries from the rat cremaster muscle and human microvascular endothelial cells (MVEC) were stimulated *in vitro* with the AMPK agonist 5-amino-4-imidazolecarboxamide riboside (AICAR; 2 mM). In MVEC and muscle resistance arteries AICAR enhanced Thr 172 phosphorylation of AMPK, Ser 72 phosphorylation of Acetyl CoA Carboxylase (ACC) and Ser 1177 phosphorylation of eNOS. 3D fluorescence microscopy showed localization of phosphorylated AMPK in the plasma membrane and in the nucleus (figure 1). In muscle resistance arteries, AICAR induced a marked vasodilatation of resistance arteries (18+5 percent diameter change, P=0.01) that was abolished by inhibition of AMPK activity with Compound C (to 3+3 percent) or inhibition of NO synthesis with N-G-nitro-L-arginine (L-NA; to −9+3 percent). To study effects of AMPK activation on insulin-mediated vasoreactivity of skeletal muscle resistance arteries, vasoreactivity to insulin (4–3400 microU/ml) was studied in the absence and presence of AICAR. In the absence of AICAR, insulin did not induce vasodilatation (0+3 percent diameter change at 272 microU/ml). In contrast, insulin induced vasodilatation in the presence of AICAR (20+5 percent at 272 microU/ml, P<0.05 vs. insulin). This vasodilatation was abolished by pretreatment with L–NA (to 3+1 percent). In contrast, insulin induced dose-dependent vasoconstriction in the absence of AICAR (−26+2 percent at 272 microU/ml), showing that AICAR inhibits vasoconstrictor effects of insulin. Western blot analysis showed that this inhibition was accompanied by an inhibition of insulin-mediated activation extracellular-regulated kinase 1/2 (ERK1/2).
Conclusion: Activation of AMPK induces NO-mediated vasodilatation of skeletal muscle resistance arteries and uncovers insulin-induced, NO-dependent vasodilatation. This interaction of AMPK with insulin-mediated vasoreactivity is achieved by inhibition of insulin's ERK1/2-dependent vasoconstrictor effects and potentiation of insulin's ERK1/2-independent vasodilator effects. These data provide a new mechanism by which exercise improves endothelial function in muscle.

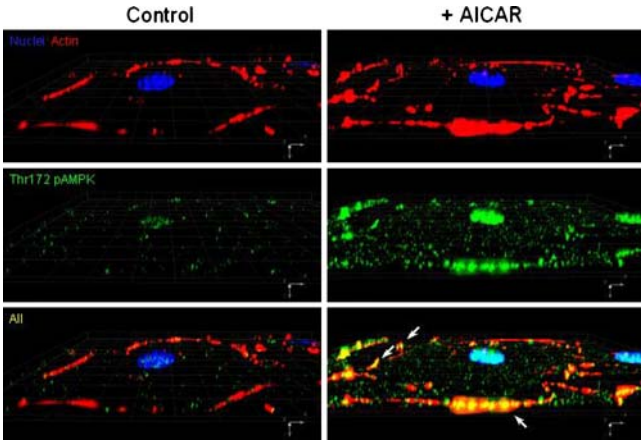

Fig 1. Intracellular localization of activated AMPK in microvascular endothelial cells

Supported by Dutch Diabetes Foundation

0128

Peroxisome-proliferator activated receptor-δ (PPARδ) mediates high glucose-induced downregulation of glucose transport in vascular endothelial cells

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Background and Aims: Increased influx of glucose to vascular endothelial cells (VEC) is closely associated with the development of cellular dysfunction and contributes to the development of macrovascular disease in diabetic patients. Yet, VEC protect their intracellular environment against such effects by reducing the rate of glucose transport when exposed to high glucose levels. It has been shown that eicosanoids, and in particular, 12-hydroxyeicosatetraenoic acid (12-HETE, the product of 12-lipoxygenase), whose production is increased in VEC exposed to high glucose, participate in this protective mechanism. Several eicosanoids are known ligands to PPAR isoforms. The present study was performed to determine whether members of the PPAR family were involved in the downregulation of glucose transporter-1 (GLUT1) expression and its plasma membrane abundance in VEC maintained at high glucose conditions.
Materials and Methods: Primary bovine aortic endothelial cell cultures were incubated at 5.5 or 25.0 mmol/l glucose up to 48 h, in the absence or presence of PPARα, -γ and -δ agonists. The capacity of these agonists to mimic the effect of high glucose levels and reduce the rate of hexose uptake, GLUT-1 expression and plasma membrane localization in

VEC was then determined using the [^{3}H]-2-deoxy-D-glucose uptake assay, cell-surface biotinylation and Western blot analysis.

Results: VEC, exposed to the high glucose level, reduced the rate of hexose uptake, GLUT-1 content and plasma membrane abundance in VEC by 40–60%, in comparison with cultures that were maintained at 5.5 mmol/l glucose. The PPARα agonist WY-14,643 and the PPARγ agonist troglitazone did not reduce the rate of hexose transport into the cells. In fact, both agonists further increased the rate of transport, indicating that they possessed the opposite effect of high glucose. However, the selective PPARδ agonist GW501516 fully mimicked the effect of high glucose and reduced the rate of hexose transport in a dose- and a time-dependent manner in VEC exposed to 5.5 mmol/l glucose. Moreover, this effect resulted from a similar decrease of total cell content of GLUT-1 and its plasma membrane localization. We then asked whether 12-HETE might be a specific ligand for PPARδ, which mediated the down-regulatory response. We exposed VEC, maintained at 25.0 mmol/l glucose, to baicalein (a selective inhibitor of 12-lipoxygenae) in the absence or presence of GW501516. In the former case baicalein prevented high glucose-dependent downregulation of hexose transport. However, in the latter case GW501516 reversed this action of baicalein and the rate of hexose transport remained low. Thus, the synthetic PPARδ agonist GW501516 substituted for 12-HETE, whose prudction was blocked.

Conclusion: This study shows that hyperglycemia-induced downregulation of hexose transport and GLUT-1 expression in VEC requires the formation of an active 12-HETE-PPARδ complex. This complex may dimerize with its cognate RXR (retinoid X receptor) to regulate the expression of specific genes involved in this regulatory mechanism. Our model is particularly attractive in view of recent studies that show a PPARδ-dependent increase of peripheral glucose utilization in various animal models. These reports and our present findings indicate that PPARδ might be a potential target for drug development, aimed at reducing hyperglycemia-induced VEC dysfunction and macrovascular disease in diabetes.

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0129

Visfatin expresses in the vascular endothelial cells and inhibits apoptosis induced by high glucose

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Background and Aims: Diabetes mellitus causes various cardiovascular complications. Hyperglycemia induces apoptosis in endothelial cells, but the precise molecular mechanisms are not yet well understood. Visfatin, also known as pre-B cell colony-enhancing factor (PBEF), was identified as a novel adipocytokine exerting insulin-mimetic effects in various insulin-sensitive tissues. Previous work has shown that PBEF is expressed in many tissues and play an antiapoptotic role in neutrophil stimulated by inflammatory stimuli. This study was to investigate the effect of high glucose on expression of visfatin in vascular endothelial cells and the role of visfatin on apoptosis induced by high glucose.

Materials and Methods: Human umbilical vein endothelial cells (*HUVECs*), were incubated in high glucose (25 mmol/L) for different time intervals. Visfatin mRNA and protein expression were measured by quantitative real-time reverse transcription-polymerase chain reaction and western blotting, respectively. To view the effect of visfatin on cell apoptosis induced by high glucose, *HUVECs* were incubated in high glucose (25 mmol/L) in the presence or absence of different doses of human recombinant visfatin with or without a special antibody to visfatin for the indicated time. For detection of apoptosis Annexin V/propidium iodide (PI) staining was used, and the enzymic activity of caspase-3, which is the final effector caspase in cytosol, was determined by colorimetric protease assay as well.

Results: Visfatin is expressed in endothelial cells. Compared with normoglycemia, exposure to hyperglycemia, but not mannitol, changed its expression at both mRNA and protein levels which exhibited a temporal increase reaching maximal levels about 4(for mRNA) or 8(for protein) hours after high glucose incubation followed by durative decrease ($P<0.05$, $n=6$). Recombinant visfatin (20–100 ng/ml) dose-dependently inhibited apoptosis in endothelial cells induced by high glucose ($p<0.05$, $n=6$), which was specific as anti-visfatin antibody(1:500) completely neutralized this effect ($P<0.01$, $n=3$). Visfatin pretreatment also resulted in reduced activation of caspase-3 reflected in reduced levels of *in vitro* enzymatic activity($P<0.05$, $n=4$).

Conclusion: Hyperglycemia could regulate visfatin expression in endothelial cells and extraneous visfatin significantly inhibited hyperglycemia-induced apoptosis in endothelial cells through reducing caspase-3 activation. These results suggest that visfatin may be a potential therapeutic target for preventing cardiovascular complications of diabetes.

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0130

Inhibition of glycogen synthase Kinase 3 beta (GSK3B) prevents palmitate-induced human umbilical vein endothelial cells (HUVEC) apoptosis

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Background and Aims: Dyslipidemia is a risk factor for diabetes-accelerated atherosclerosis. Free fatty acid such as palmitate have been reported to induce failure of endothelial cells function and to increase endothelial cells apoptosis. GSK3β as a serin/threonine kinase, plays a central role in neuronal cells death. However, the role of GSK3β on the death of endothelial cells is unclear. In this study, we investigated whether GSK3β plays any role in palmitate-induced HUVEC apoptosis
Materials and Methods: Palmitate induced HUVEC death was measured by DNA ladder, DAPI and MTT assay. To investigated the mechanism of HUVEC death by palmitate, we used several inhibitors and adenovirus of kinase death and constitutively active form of GSK (GSK-S9A: 9th serine switched to alanine).
Results: Incubation of HUVEC with palmitate significantly increased dephosphorylation of GSK3β at 9th serine and also increased apoptotic features such as activation of caspases 3 and 9 , release of cytochrome c and poly-ADP ribose polymerase (PARP) cleavage. Blocking GSK3β activity with selective inhibitor (TDZD-8, lithium, SB216763) significantly prevented activation of palmitate-induced caspase 3 and 9, PARP cleavage and HUVEC apoptosis. To further assess the role of GSK3β in palmitate-induced apoptosis, HUVEC were infected with adenovirus of kinase-inactive GSK3β and these cells resisted palmitate-induced apoptosis.
Conclusion: These results therefore suggest that the prevention of palmitate induced endothelial cells apoptosis through GSK3β and GSK3β represents a novel target for therapeutic agents.
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0131

Rosiglitazone reverses the alterations of capillary permeability but does not improve nerve function in Zucker Diabetic Fatty rats

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Background and Aim: An increase in capillary permeability is a common disorder in diabetes and may play a role in the pathophysiology of peripheral neuropathy. Several data suggest that glitazones exert beneficial effects on endothelial function but may induce edema. The aim of the present study was to test the effect of rosiglitazone on the capillary filtration of albumin (CFA) and peripheral nerve function in ZDF rats.
Materials and Methods: Fifteen ZDF rats were treated by rosiglitazone from 2 to 6 months of age, and were compared to 15 untreated ZDF rats and 15 lean (fa/?) controls. CFA and lymphatic function were evaluated by a noninvasive isotopic test using technetium-labelled albumin at 2 and 6 months. Sensory and motor nerve conduction velocities were measured at 6 months.
Results: At 2 months CFA was significantly higher in ZDF rats than in lean controls ($p<0.04$). At 6 months, as compared to lean controls, CFA was markedly higher in untreated ZDF rats ($p<0.001$) but very similar in rosiglitazone-treated rats. Lymphatic function was similar in ZDF rats and controls at 2 months but significantly impaired in ZDF rats at 6 months, whatever treated or not treated ($p<0.001$). Motor and sensory nerve conduction velocities were significantly impaired at 6 months in both treated and untreated ZDF rats as compared with controls.
Conclusion: The present data show that in ZDF rats, 1) rosiglitazone prevents the increase in capillary permeability but not the impairment in lymphatic function, which suggests that alteration of lymphatic function might be involved in induction of edema during rosiglitazone treatment ; 2) rosiglitazone does not prevent the impairment in nerve function.
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0132

Insulin resistance and postprandial endothelial dysfunction: Does attenuation of postprandial hyperglycaemia make a difference?

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Background and Aims: Postprandial hyperglycemia is an independent risk factor for cardiovascular disease, even before the development of overt diabetes. We aimed to determine if attenuation/normalization of postprandial hyperglycemia (with nateglinide administration — an oral antidiabetic of ultra-rapid action that stimulates postprandial first-phase insulin secretion) could diminish endothelial dysfunction, one of the earliest markers of atherosclerosis.
Materials and Methods: We assessed post-oral glucose load endothelial function in 56 insulin-resistant subjects with impaired glucose tolerance (IGT) and with the following baseline characteristics: (mean values±SEM) Homeostasis Model Assessment (HOMA) 6.41±0.7, body mass index (BMI) 34.4±5.68 kg/m, Oral Glucose Tolerance Test (OGTT): 0 min 6.2±1.0 mmol/l and 120 min. 11.9±2.7 mmo/l, HbA1C 6.4±0.82%, waist circumference 115.8±20.5 cm, age 57.5±7.3 years. All subjects were randomized to either intervention or time control group. In addition, subjects in the intervention group received an individually adjusted dose of nateglinide on a second day (Day 2), 10 min. before oral glucose load. Furthermore, we examined post-oral glucose load endothelial function in 9 healthy, insulin-sensitive subjects (HOMA 0.65±0.13, BMI 23.20± 2.13 kg/m^2; OGTT 0 min 4.12±0.36 and 120 min. 5.58±0.95 mmol/l, waist circumference 80.77±2.93, age 41.11±3.02). Flow-mediated dilation (FMD) technique was used to measure endothelial function.
Results: The following postprandial Delta FMD values (fasting FMD value subtracted from postprandial FMD value) were seen in intervention and control groups respectively (mean value±SEM) : (−3.42±1.67%) p=0.04 and (−2.20±1.19%) p=0.07. The Delta postprandial FMD value in the healthy group was 4.80±1.41, p=0.009. On Day 2, when nateglinide was administered 10 min. prior to glucose-load in the intervention group, Delta FMD values increased to 2.32±1.45%, p=0.02.
Conclusion: Endothelial function in insulin resistant individuals deteriorates after an oral glucose load (which represents a standard meal). Attenuation of post load hyperglycemia not only nullifies this negative effect, but also enhances endothelial function to the extent that it mimicks post glucose-load endothelial function in healthy. These findings could substantially impact the prevention of cardiovascular disease in insulin resistant individuals with pre-diabetes or early type 2 diabetes.
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OP 23

Experimental genetics: regulation of insulin secretion

0133

Distinct diabetes susceptibility loci impair insulin exocytosis and glucose metabolism in pancreatic beta cells

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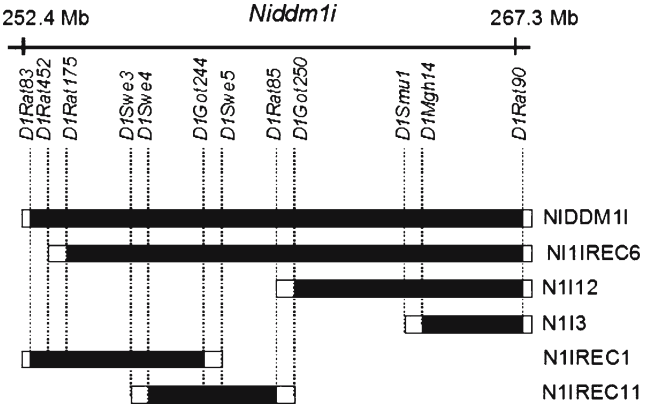
Background and Aims: Genetic factors are important in the development of type 2 diabetes. Coupling of candidate genes to cellular functional defects in type 2 diabetes is facilitated by studies of inbred animal models with inherited susceptibility to the disease. The *Niddm1i* locus, a major glucose-regulating locus in the diabetic GK rat, has previously been coupled to defect insulin secretion in pancreatic β-cells. Interestingly, this locus corresponds to a diabetes susceptibility locus on human chromosome 10q24–26. Here, to further fine-map the *Niddm1i* locus, we have generated a series of subcongenic strains harbouring different parts of the *Niddm1* and investigated their insulin release properties.
Materials and Methods: Transfer of GK alleles onto the genome of F344 rats by repeated backcrossing established the homozygous congenic strain F344.GK-*Niddm1i* (NIDDM1I) and the subcongenic strains were established in a similar way by backcrossing NIDDM1I onto F344 rats. In vivo hormone release, islet morphology, pancreatic RNA and protein content measurements, capacitance measurements, mitochondrial potential measurements, and ATP/ADP assays were applied to the congenic strains.
Results: All congenic strains displayed normal insulin content and β-cell mass. Glucose-evoked insulin secretion, as studied in batch-incubated islets, was 61% lower in the NIDDM1I strain (with GK-derived genome in the entire 16 Mb *Niddm1i*-region) compared to control rats ($p<0.001$). The insulin secretion in N1IREC1, harbouring 4 Mb GK genome proximal in *Niddm1i*, was suppressed by 54% ($p<0.001$), and N1I12, harbouring 8.7 Mb GK genome distal in *Niddm1i* (no overlap with N1IREC1), showed a 47% reduction in insulin secretion upon glucose stimulation ($p<0.001$). Both NIDDM1 and N1I12 showed a 50% reduced insulin release ($p<0.05$) in response to depolarization with high potassium, whereas the response of N1IREC1 to high potassium was intact. In excellent agreement with the islet insulin release measurements, single β-cell exocytosis was reduced by 45% in NIDDM1

(p<0,05) and by 49% in N1I12. Interestingly, the N1IREC1 strain showed a 35–50% enhanced exocytotic capacity compared to NIDDM1I (p<0.05) and N1I12 (p<0.01).
Since the increase in ATP/ADP ratio is fundamental to initiation of β cell electrical activity and insulin secretion, we also examined the cytosolic adenine nucleotide levels. N1IREC1 islets showed a markedly lower ATP/ADP ratio (30–40%, p<0.01) compared to control, NIDDM1 and N1I12. Furthermore, N1IREC1 demonstrated an increased mitochondrial hyperpolarization compared to the other strains upon glucose addition (p<0.05).
Conclusion: 1. A 3 Mb locus located proximally in *Niddm1* encodes for defective glucose metabolism in the β-cells.
2. A 4.5 Mb locus in the distal part of *Niddm1* generates a more profound defect in insulin exocytosis.
3. A few highly interesting candidate genes exist in both loci.



Supported by the European Foundation for the Study of Diabetes

0134

Global analysis of gene expression profiles reveals both shared and distinct roles of Hnf4α

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Background and Aims: Mutations in HNF4α and HNF1α (also known as *TCF1*) transcription factor genes cause forms of MODY diabetes that are closely related based on clinical or other phenotypic criteria. This suggests that the two genes may interact functionally in beta cells. It is known that Hnf1α is a regulator of Hnf4α in pancreatic islets, and human genetic evidence suggests that Hnf4α may regulate Hnf1α. However, the precise manner in which these two transcriptional factors interact in beta cell transcriptional regulatory networks is still unknown.
Materials and Methods: Pancreas-specific Hnf4α deficient mice (pHnf4αKO) were generated by crossing Pdx1-Cre with Hnf4α-loxP mice. Greater than 85% inactivation was verified by real-time PCR of Hnf4α mRNA in isolated islets. Expression profiles of cultured islets from control, pHNF4αKO, and Hnf1αKO mice were analyzed with Affymetrix chips. Statistical analysis of the data was carried out with SAM, and genes exhibiting differential regulation at a 5% false discovery rate were selected.
Results: The absence of Hnf4α in mouse pancreas did not result in diabetes or impaired intraperitoneal glucose tolerance. Blood glucose in fed mice was mildly but significantly decreased. Despite this lack of severe phenotypic consequences, expression analysis of pHnf4αKO islets revealed significantly increased or reduced regulation of 7% of islet genes. This regulatory role of Hnf4α affected several key functional categories. Interestingly, while many genes were uniquely regulated in either pHNF4αKO or Hnf1α-deficient islets, nearly 30% of genes exhibiting more than 2-fold reduction in pHNF4αKO were also down regulated>2-fold in Hnf1α-deficient islets. This overlap varied among different functional categories, with some gene classes exhibiting shared regulation in 100% of genes. These results could not be explained solely by the fact that Hnf1α regulates Hnf4α, suggesting the existence of complex regulatory interactions between these genes.
Conclusion: These results provide new insights to understand the role of Hnf4α in pancreatic islets. They also demonstrate a shared gene expression signature of Hnf4α and Hnf1α deficient-islets, in keeping with the common phenotype during the diabetic stage of MODY1 and MODY3. This framework will allow us to gain an in depth understanding of a key regulatory interaction between two genes involved in human diabetes.

0135

Mice with conditional beta-cell knockout of glutamate dehydrogenase exhibit severe insulin secretion impairment

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Background and Aims: Glutamate dehydrogenase (GDH) is a mitochondrial enzyme that can reversibly form glutamate from the TCA cycle intermediate alpha-ketoglutarate using NAD(P)H as coenzyme. The importance of GDH for the control of insulin secretion is well recognized, although its specific role is still debated. Accordingly, beta-cell specific abrogation of GDH in a mouse model would allow unprecedented investigations of this critical pathway.
Materials and Methods: We recently generated transgenic mice with tissue specific conditional abrogation of GDH using the lox/P-flp-recombinase strategy. The targeting vector carried Glud-1 exon 7, comprising the NADH binding site essential for GDH activity, flanked by two lox/P sites. The construct was introduced into mouse ES cells by electroporation. Selected recombinant ES clones were screened by PCR and Southern blot to check for the occurrence of homologous recombination before proceeding to mouse blastocyst injection. The founder mice were crossed to obtain F1 mice carrying a mutant Glud-1 genomic locus containing the exon 7 flanked by lox/P sites and the neoR-cassette. Next step was the excision of the neoR-cassette by crossing the F1 with Flp deleter mice. The mice generated this way were crossed with mice expressing the Cre recombinase under the control of an insulin promoter (RIP), resulting in the knockout of GDH exon 7, specifically in beta-cells, thereby leading to the abrogation of GDH activity.
Results: Immunohistochemistry on pancreas sections revealed disorganized islets in homozygous GDH beta-cell specific knockout mice. Specifically, alpha-cells were present in the core of the islet, normally composed essentially of beta-cells. GDH beta-cell knockout mice did not show any difference in body weight compared to wild type mice. Interestingly, homozygous mice appeared to be normoglycaemic despite markedly reduced blood insulin levels at the fasting state (7 weeks: −56%; 12 weeks: −61%). Glucose tolerance during an ipGTT was also normal, again accompanied by marked reduction in plasma insulin levels 15 min after glucose injection (7 weeks: −45%; 12 weeks: −47%). In situ pancreatic perfusions confirmed that knockout mice exhibited severe impairment of glucose stimulated insulin release compared to control mice. The secretory responses were reduced both during the first phase (−51%, $p<0.005$) and the second phase (−48%, $p<0.05$). Ongoing studies investigate the susceptibility of these mice to diet-induced obesity and diabetes. Preliminary results showed that homozygous knockout mice challenged during 15 weeks with a high fat diet did not become obese compared to control mice. They were also able to maintain normoglycaemia as opposed to hyperglycaemic controls.
Conclusion: Based on our recently generated beta-cell specific GDH null mice, present data show the importance of GDH in the control of insulin secretion. The results also indicate unexpected adaptative capacity of the organism to maintain normoglycaemia despite severe beta-cell dysfunction.
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0136

GPR40 knock-out mice do not develop obesity induced glucose intolerance

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Background and Aims: GPR40 is a G-protein coupled receptor that is highly expressed in pancreatic beta cells and is activated by medium and long-chain free fatty acids (FFA). Acute FFA-induced insulin secretion has been shown to be dependent on GPR40, however, it is unclear whether GPR40 is also involved in mediating detrimental effects of chronic fatty acid exposure on beta-cell function. In order to elucidate the role of GPR40 to chronic exposure of elevated levels of FFA, we characterized GPR40 knock-out mice fed a high-fat diet and investigated their islet function *in vitro*.
Materials and Methods: Male GPR40 knock-out mice (n=6) and wild type littermates (n=6) were fed a high-fat diet (Research Diets D12492) for 15 weeks. Bodyweight and food consumption were monitored weekly, fasted insulin and glucose monthly. An oral glucose tolerance test (OGTT) was performed after 15 weeks on the diet. In vitro insulin secretion was measured from batches of 3 islets isolated by collagenase treatment.
Results: After 15 weeks of high-fat feeding male GPR40 knock-out mice showed a significant lower glucose excursion combined with a trend of lower insulin response during OGTT compared to their control littermates. GPR40 knock-out mice had lower fasted insulin ($p=0.04$) but not glucose levels in comparison to control animals. In vitro characterization of GPR40 knock-out islets isolated from the mice after the feeding experiment showed that glucose induced insulin secretion was comparable to wild type islets but acute fatty acid-enhanced insulin secretion was abolished. In order to investigate if GPR40 is involved in long-term fatty acid induced impairment of beta cell function, glucose stimulated insulin secretion was analysed after incubation of GPR40 knock-out and wild type islets for

48 h with either 400 μM palmitic acid or oleic acid. Exposure of wild type islets to either fatty acid resulted as expected in a reduction of glucose-induced insulin secretion. However, in contrast to a previous report GPR40 knock-out islets were not protected from long-term inhibitory effects of fatty acids.

Conclusion: Our *in vivo* data on GPR40 knock-out mice suggest that loss of GPR40 leads to decreased hyperinsulinemia, which results in increased insulin sensitivity. The data also shows that *in vitro* GPR40 mediates only acute effects of FFA on insulin secretion but is most likely not involved in FFA induced islet lipotoxicity.

0137

Sterol regulatory element binding protein1 (SREBP1) is required for the induction by glucose of β-cell genes involved in glucose sensing

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Background and Aims: Culture of mouse islets at elevated glucose concentrations leads to the enhanced secretion of insulin at both low and high glucose concentrations. In a recent study using islets from control mice or mice deleted for the sterol regulatory element binding protein1 (SREBP1) gene, we found that the latter transcription factor, a "master" controler of lipogenesis, was required for this response. Here, we have examined the changes in the expression of β-cell specific genes associated with insulin hypersecretion and the role of SREBP1 in regulating the expression of each one.

Materials and Methods: Islets from 12–16 week old SREBP1$^{-/-}$, SREBP1$^{+/-}$, or wild-type C57BL/6 mice were isolated by collagenase digestion and handpicking. After isolation, islets were incubated for 16 h at 11 mmol/l glucose, and then cultured for 96 h at 8 or 30 mmol/l glucose before measuring glucose-stimulated insulin secretion and gene expression. Secreted insulin was measured after static incubation at the indicated glucose concentration for 30 min., by radioimmunoassay. SREBP1c, fatty acid synthetase (FAS), acetyl-CoA carboxylase-1 (ACC1), glucose transporter 2 (Glut2), glucokinase (Gk), sulfonylurea receptor 1 (SUR1), potassium inward rectifier 6.2 (Kir6.2), pancreatic-duodenal homeobox factor-1 (Pdx1), Nkx6.1, and neurogenin3 (Ngn3) mRNA levels were determined after RNA extraction in Trizol (Sigma) and quantitative real-time RT-PCR (Taqman™) and were normalised to cyclophilin A levels which were not different between groups. Data from three mice per condition are quoted as means±S.E.M. Statistical comparision was by ANOVA and Newman Keuls test.

Results: Cultured at 8 mmol/l glucose, islets from SREBP1$^{-/-}$ mice displayed decreased levels of FAS (63%, $p<0.05$) and Gk (41%, $p<0.05$) mRNAs, but increased Ngn3 mRNA (2.19±0.35-fold, $p<0.05$) compared to wild-type littermates. Islets from wild-type mice chronically (96 h) exposed to elevated (30 *versus* 8 mmol/l) glucose concentration displayed a 5–10-fold increase in both basal and glucose (16 *versus* 3 mmol/l)-stimulated insulin release. This change was associated with increased levels of mRNAs encoding SREBP-1c (1.37±0.13-fold, $p<0.05$), FAS (2.01±0.35-fold, $p<0.05$), ACC1 (2.65±0.45-fold, $p<0.05$), Glut2 (2.05±0.37-fold, $p<0.05$), Gk (2.46±0.82-fold, $p<0.05$), Pdx1 (3.58±0.60-fold, $p<0.05$), SUR1 (3.18±0.63-fold, $p<0.05$), Kir 6.2 (2.99±0.50-fold, $p<0.05$), Ngn3 (2.33±0.14-fold, $p<0.001$) and Nkx 6.1 (3.65±0.81-fold, $p<0.05$). With the exception of FAS mRNA which was still induced by culture at 30 mmol/l glucose in islets from SREBP1$^{-/+}$ mice, the up-regulation by high glucose of each of the above genes was completely abolished in islets from mice deleted for one or both SREBP1 allele.

Conclusions: These results demonstrate a requirement for SREBP1 in the hypersecretion of insulin resulting from chronic exposure to high glucose concentrations. In addition to the requirement for SREBP-1 in the induction of lipogenic genes, SREBP1 is also, unexpectedly, necessary for the up-regulation of genes directly involved in the control of the β-cell transcriptome (Pdx1) and in glucose sensing (Glut2, Gk, Kir6.2, SUR1). Induction of SREBP-1c may therefore be involved in the adaptive insulin hypersecretion observed in hyperglycemic states such as insulin resistance and pregnancy.

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0138

Beta-cell function is improved in RIP-SOCS-3 transgenic mice fed normal and high-fat diets

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Background and Aim: Suppressor of cytokine signaling 3 (SOCS-3) regulates pancreatic β-cell mass and proliferation. SOCS-3 is induced by leptin and inhibits leptin-mediated JAK/STAT signaling in β-cells. Leptin has been demonstrated to inhibit insulin secretion and insulin gene

expression. Dysregulation of the adipoinsular axis may contribute to hyperinsulinemia and insulin resistance. High-fat diet (HFD) results in obesity and insulin resistance and glucose intolerance due to insufficient islet compensation to increased insulin demands, and leptin levels are elevated in parallel with increased body weight (BW). The aim of this study was to investigate *in vivo* and ex vivo effects of overexpression of SOCS-3 on β-cell function in normal mice and in mice with HFD-induced insulin resistance.

Material and Methods: Transgenic mice (tg) with β-cell specific overexpression of SOCS-3 (SOCS3-RIP-Tg) were generated. At 8 weeks of age, female wildtype (wt) and SOCS-3 tg mice were fed HFD (58% fat by energy) for 12 weeks. Control wt and tg mice were fed a normal diet (ND) with 11% fat. Islet function was studied *in vivo* by intravenous glucose tolerance test (IVGTT) and intravenous arginine challenge. Islets were isolated and glucose-stimulated insulin secretion (GSIS) was measured. Western blots confirmed the overexpression of SOCS-3 in tg islets

Results: SOCS-3 expression was induced in islets from wt mice fed the HF-diet. BW gain was similar in SOCS-3 tg mice and in wt mice in both diet groups. There was no difference in fasting plasma glucose while insulin levels were slightly reduced in SOCS-3 tg fed HFD. Tg mice on ND had improved insulin response to glucose in the IVGTT (1 min peak value: 2250±340 vs. 1460±190 pM, $P<0.05$), which resulted in improved glucose elimination (K_G: 2.6±2.1 vs. 1.3±0.2%/min, $P=0.002$). Glucose tolerance was similar in the HFD-fed wt and tg mice. Insulin secretion *in vitro* was augmented 4.6 fold with 11.1 mM glucose compared to 2.8 mM glucose in both wt and tg islets from ND-fed mice ($P<0.01$ in both groups). GSIS was impaired in wt HF-diet fed mice while the response was restored in SOCS-3 tg islets (1.3±0.3 fold increase in HFDwt vs. 3.2±0.7 fold increase in HFDtg, $P<0.05$). The islet response to intravenous arginine was similar in wt and tg mice on both diets.

Conclusions: SOCS-3 improved β-cell function *in vivo* and ex vivo in SOCS-3-RIP-tg mice fed ND, and restored HFD-induced impaired GSIS ex vivo. We hypothesise that the *in vivo* increased response to glucose and improved glucose elimination observed in SOCS3 tg mice on ND is masked in SOCS-3 tg mice on HFD, since HF-feeding in itself stimulates islet expression of SOCS3. We suggest that increased expression of SOCS-3 in islets is involved in the islet adaptation in high-fat diet induced glucose intolerance.

OP 24

Fetal programming

0139

Compromised beta-cell development and reduced glucokinase expression in weanling offspring from dams maintained on a high fat diet during gestation

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Background and Aims: A high fat diet (HFD) is known to adversely affect beta-cell development and function evident by reduced beta-cell mass, hyperglycemia and hyperinsulinemia. Glucokinase (GK) is the key glycolytic enzyme involved in glucose stimulated insulin secretion from beta-cells. An insult *in utero* is believed to programme progeny metabolically predisposing them to develop Type 2 diabetes in adulthood. Our previous data have shown that dams fed a HFD during gestation produced weanlings that were hyperglycemic and hypoinsulinemic. The aim of the present study was to investigate whether the HFD also affected beta- and alpha-cell development. In addition, we sought to establish whether the HFD altered expression of GK.

Materials and Methods: Weanling offspring were obtained from dams maintained on a HFD (2.06 kcal/g: 40% of mainly saturated fat as energy, 14% protein and 46% carbohydrate) for either the first (HF1), or second (HF2), or third (HF3) week of gestation, or throughout (HF-G) gestation, followed by a standard laboratory diet (2.60 kcal/g: 10% fat, 15% protein and 75% carbohydrate) for the duration of lactation. At postnatal day 21, pancreata were excised and either snap-frozen for quantitative PCR (of GK mRNA expression) or processed for immunocytochemical examination and image analysis (to determine beta- and alpha-cell volume, number and size and the levels of GK immunoreactivity).

Results: HF1, HF2 and HF3 weanlings displayed a reduction in beta-cell volume, number and size. In addition, the alpha-cell sizes were also smaller in these weanlings. However, the alpha-cell volume and number was not affected by the HFD. Relative quantification revealed a decrease in GK mRNA expression in HF1, HF2 and HF3 weanlings. This was supported by reduced immunoreactivity for GK in these weanlings. In contrast, HF-G weanlings had no significant differences in beta-cell volume, number or size. Alpha-cell development also appeared to be normal with no changes also detected in either GK mRNA expression or immunoreactivity.

Conclusion: Maintenance of dams on a HFD, for any single week of gestation, has a deleterious effect on the

beta-cell development of their weanling offspring. In contrast, offspring from dams fed a HFD throughout gestation showed no significant changes in islet cell development. Switching the maternal diet during gestation appears to programme weanling progeny inducing diabetogenic effects characterized by compromised beta-cell development and reduced GK expression.

0140

Effects of maternal obesity on metabolic syndrome in the offspring

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Background and Aims: Epidemiological studies have shown that offspring born to women that have been starved during pregnancy in the Second World War in the Netherlands, often had a low birth-weight and higher risk of developing metabolic syndrome (diabetes, obesity, high blood-pressure). This suggests that there are prenatal environmental factors involved in development of the metabolic syndrome.
Not only starvation, but also maternal obesity may be important for the predisposition to the metabolic syndrome in the offspring. In ethnic groups with a high incidence of diabetes and obesity, like the Pima indians, children from diabetic mothers have a higher risk of becoming diabetic compared with children from non-diabetic mothers. This effect is much stronger with maternal diabetes compared with paternal diabetes, suggesting a non-genetic mode of transfer to the offspring.In this project, the effects of maternal obesity on the development of the metabolic syndrome in the offspring were investigated in rats.
Materials and Methods: Maternal obesity was induced by i3vt infusion of the melanocortin 34 antagonist SHU9119 during the last two weeks of pregnancy and the first two weeks of lactation. Male offspring rats from these pregnancies were used at the age of 3–4 months to assess the risk of metabolic syndrome.
Results: Body weight was increased in offspring from SHU mothers, starting from 68 days of age. Food intake was not changed, but water intake was increased in SHU offspring. Respirometric measurements showed increased RQ and a trend to decreased energy expenditure in the dark phase. This was associated to decreased locomotor activity in the dark phase. Oral glucose tolerance tests showed an increase in basal glucose, with no difference in insulin levels, suggesting glucose intolerance in SHU offspring.
Conclusion: We conclude that maternal obesity increases the risk of the metabolic syndrome in the offspring in a non-genetic way. This is mainly due to changes in fat and glucose metabolism, and decreased physical activity, resulting in high body weight and glucose intolerance.
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0141

Intrauterine growth retardation leads to the functional change of insulin secretion in the newborn rat

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Background and Aims: Embryonic pancreatic development had a close relation to adult diseases. Intrauterine growth retardation (IUGR) is a common complication of pregnancy and a risk factor for both perinatal disease and disorders of later life, especially impaired glucose tolerance and diabetes. The aim of this study is to investigate the functional change of insulin secretion in IUGR newborn rat and explore the mechanism of reduced blood insulin level.
Materials and Methods: SD pregnant rats were divided into two groups: control group and intrauterine energy restriction group. The animal model of IUGR in rats was made by 50% calorie restriction in pregnant rats from gestational day 15 until term. Pancreata of control and IUGR newborn rats were dissected respectively. After abstraction of totle RNA, RT-PCR was used to study the expression of genes related to insulin synthesis and exocytosis. Intraperitoneal glucose tolerance tests(IPGTT) and ELISA were done to detect the function of pancreatic islet.
Results: Birth weight and pancreas mass of IUGR newborn rats were significantly lower than those of controls. Although no significant differences were observed in genes expression of insulin and PDX-1, the expressions of genes related to insulin exocytosis such as munc13-1, vamp-2, syntaxin1a, rab3a were reduced markedly in IUGR newborn rats. The blood insulin level and insulin secretion response to glucose challenge were also reduced simultaneously in IUGR newborn rats compared with normal newborn rats.

Conclusion: IUGR can induce changes in glucose homeostasis in newborn rats. The main cause of this abnormality in the process might be the reduced function of insulin exocytosis.
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0142

Diminished beta-cell neogenesis and proliferation contribute to reduce beta-cell mass in neonatal undernourished rats

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Background and Aims: We have previously shown that Wistar fetuses from protein caloric undernourished pregnant rats (35% of control diet during the last week of pregnancy) at 21 days post coitum (dpc) exhibit increased beta-cell mass. This alteration can be related to the stimulation of beta-cell proliferation induced by locally increased IGF-1 in pancreas. On the contrary, continuation of maternal food restriction caused reduced beta-cell mass in undernourished (U) neonates at 4 days of life, representing only 58% of control values. This effect persists until adult age. During pancreas development, new beta-cells can be formed either by replication of pre-existing differentiated beta-cells or by neogenesis (differentiation from stem-cells) and the loss of beta-cells can occur by apoptosis. The aim of this study was to investigate whether a condition of undernutrition affects both beta cell proliferation and differentiation in U rats on postnatal day 4 and the implication of the IGF system, highly responsive to nutritional status, in these processes. To this end we have evaluated 1) replication and differentiation of beta-cells in pancreas, 2) serum IGF-1 and -2 levels and 3) IGF-1 and -2 protein content in liver and pancreas.
Materials and Methods: Food restricted pregnant rats received 35% of control diet since the last week of gestation. Spontaneous beta-cell regeneration and beta-cell replication were evaluated by immunocytochemistry and morphometry. Serum concentrations of IGF-1 and IGF-2 were measured by radioimmunoassay and radioreceptor assay respectively. The protein content of IGF-1 and -2 was analyzed by Western blot in pancreas and liver.
Results: The results show that both beta cell replication and beta cell neogenesis were markedly decreased in U rats at 4 days of life. Serum IGF-2 levels as well as liver and pancreatic protein content were found increased in U rats, whereas the serum concentration and abundance of IGF-1 protein in pancreas and liver were decreased. All values were compared to those of controls.
Conclusion: Our data suggest that in U rats at 4 days of life 1) the decreased beta-cell mass found could be related to the inhibition of both beta cell proliferation and beta cell neogenesis; in which, 2) low serum IGF-1 levels together with a reduced pancreatic IGF-1 protein content could play a crucial role, and 3) in contrast to other animal models in which a reduced pancreatic expression of IGF-2 was related to a decreased beta-cell mass and to an enhancement of apoptosis, the enhanced pancreatic IGF-2 levels in the present U model could let us assume that malnutrition does not affect apoptosis in our conditions. Therefore, understanding differences in the relationship between the IGF system and the process of beta-cell growth may lead to therapeutic and preventive interventions in diabetic patients with previous episodes of undernutrition.
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0143

The effects of undernutrition on beta-cell development require a functional glucocorticoid receptor in pancreatic precursor cells

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Background and Aims: Low birth weight is strongly predictive of hypertension, cardiovascular diseases, obesity, insulin resistance and diabetes. The hypothesis of a primary defect in beta-cell development was initially studied in a rat model of maternal General Food Restriction (GFR), leading to impaired glucose tolerance at adult age. GFR caused maternal and foetal glucocorticoid (GC) elevation, which, in turn, caused a reduction in beta-cell mass in the foetus. Conversely, low GC levels were associated with increased beta-cell mass when nutrient supply is normal. In line with this, the selective inactivation of the glucocorticoid receptor (GR) gene in pancreatic precursor cells (GR-Pdx-Cre mice) was associated with a 2-fold increased beta-cell mass in adult mice while its inactivation in insulin-expressing cells (GR-RIP-Cre mice) had no consequences.
The aim of the present study were 1) to determine if the effects observed on the mutants were postnatal or antenatal,

2) to investigate if all the effects of undernutrition on beta-cell development involve the glucocorticoid receptor gene activation and 3) to decipher the cellular mechanisms involved, using the GR mutant mouse models.

Materials and Methods: GR-lox/lox female mice crossed with GR-lox/lox-Pdx-Cre male mice and carrying half GR-lox/lox-Pdx-Cre and half GR-lox/lox foetuses were either nourished ad libitum (AL) or given 50% food restriction (GFR group) from E11.5 until E18.5. A similar protocol was conducted with the GR-RIP-Cre mouse line. Maternal food intake and body weight were monitored daily. At E18.5, the dams were i.p. injected with 50 mg/kg BrdU and the foetuses were excised 2 h later, weighed and the pancreas were dissected. Beta-cell fraction was analysed by morphometrical measurements after immunohistochemistry for insulin. Double immunohistochemistry for insulin and BrdU was used to measure beta-cell proliferation rate.

Results: As pregnancy progresses AL dams gained weight regularly while the body weight from GFR dams remained stable. No differences in body weight were observed between normally nourished GR-lox/lox, GR-Pdx-Cre or GR-RIP-Cre foetuses. Irrespective of the genotype, foetal weight was decreased 33% in GFR compared with AL foetuses (0.70±0.13 g vs 1.04±0.17 g, $p<0.001$).

When comparing AL foetuses, the inactivation of the GR in precursor cells (GR-Pdx-Cre) led to a 2-fold increased beta-cell fraction ($p<0.01$) and beta-cell proliferation rate ($p<0.05$) compared with GR-lox/lox foetuses, while GR inactivation in beta cells (GR-RIP-Cre) had no consequences. General Food Restriction decreased beta-cell fraction in GR-lox/lox and GR-RIP-Cre, compared to their corresponding AL-fed foetuses, while GFR-GR-Pdx-Cre and AL-GR-Pdx-Cre foetuses showed similarly elevated beta-cell fractions.

Conclusion: The absence of GC signaling in pancreatic cells favours beta-cell mass expansion and proliferation in the foetus, suggesting that GC negatively regulate these process antenatally. The cellular targets for physiological or excess circulating GC concentrations are Pdx-1-expressing precursor cells. Our results strongly suggest that all the effects of undernutrition on beta-cell development involve a functional Glucocorticoid Receptor in pancreatic precursor cells.

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0144

Non-genetic association between birth weight and insulin resistance in twins

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Background and Aims: Several studies have show an association between low birth weight and increased risk of impaired glucose tolerance, insulin resistance and type 2 diabetes later in life. Whether this association is due to a common genotype resulting in both low birth weight and metabolic abnormalities or a result of a prenatal environmental insult is still unknown. Twin studies represent a tool in investigating whether these associations between birth weight (BW) and measures of glucose metabolism are of genetic or non-genetic origin.

Materials and Methods: An oral glucose tolerance test (OGTT) was performed in a population-based cohort of twins including 142 same-sex monozygotic (MZ) and 214 dizygotic (DZ) twin pairs aged 55–73 years. Information on BW was obtained from original midwife records. Intra-twin-pair correlations (correlations between within-twin-pair differences) and multiple regression analyses with within-twin-pair differences with adjustment for BMI were performed.

Results: We found a non-genetic negative association between BW and fasting insulin, HOMA–IR and index for whole body insulin sensitivity illustrated by significant intra-twin-pair correlations among MZ twins (fasting insulin: $r=-0.27$ $p=0.001$, HOMA–IR: $r=-0.26$ $p=0.002$ and whole body insulin sensitivity index: $r=0.23$ $p=0.007$). The equivalent correlations in DZ twins were non-significant. Furthermore, a non-genetic negative association was found between BW and fasting plasma glucose data in MZ twins ($r=-0.19$ $p=0.023$). The association between 120 min post OGTT plasma glucose levels and BW was significant in both MZ and DZ twins (MZ: $r=-0.27$ $p=0.001$, DZ: $r=-0.19$ $p=0.004$).

After adjustment for BMI the association between BW and HOMA–IR, fasting plasma glucose and 120 min plasma glucose, respectively, remained significant. A 100 g decrease in BW increased HOMA–IR with 3% and 120 min plasma glucose with 2.5% in MZ twins and DZ twins, while fasting plasma glucose increased with 1.4% in MZ twins. The impact of BW on fasting insulin and whole body insulin sensitivity index, however, were not significant after adjustment for BMI.

Conclusion: We demonstrate a non-genetic negative association between BW and insulin resistance supporting an impact of the fetal environment on insulin resistance in elderly twins, independent of current body size. In addition, glucose intolerance is associated with BW, although a genetic association can not be excluded.

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OP 25

Insulin therapy

0145

Equivalence of basal insulin glargine versus prandial insulin lispro for glucose control in type 2 diabetes patients on oral agents – results of the APOLLO study

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Background and Aims: One option to control blood glucose (BG), to delay or prevent complications associated with type 2 diabetes (T2DM), is combination therapy with oral antidiabetic agents (OADs) +insulin. However, there is debate as to whether it is more beneficial to treat patients by targeting postprandial glucose (PPG) levels with a short-acting insulin, or to target fasting BG (FBG) levels with a basal insulin+OADs.

Materials and Methods: We present the results of *a* 44-week, *p*arallel, open, randomized, multinational study showing the first direct comparison of the efficacy and safety of an *O*AD regimen+either once-daily insulin glargine (n=174) or mealtime insulin *l*ispro (n=174) in T2DM patients failing *o*ral treatment (APOLLO).

Results: HbA_{1c} significantly improved, reaching target levels<7% in both groups (Table). Equivalence was established for glargine vs lispro, with the confidence interval (CI) of the mean HbA_{1c} decrease within the pre-established 0.4% limit for non-inferiority (0.157; 95% CI=0.35–0.05 [per protocol set]). Glargine provided significantly better control of FBG ($p<0.0001$) and nocturnal BG ($p=0.0017$; Table); lispro gave better PPG control, particularly after lunch and dinner (both $p<0.0001$). The mean number of overall hypoglycaemic events was significantly lower with glargine vs lispro (5.4 vs 24.4 events/patient-year). Daily insulin doses at endpoint did not differ with glargine vs lispro (42.1±25.9 vs 45.1±25.6 IU).

Conclusion: A regimen using the long-acting insulin analogue glargine plus OADs to target FBG offers glucose control equivalent to that with the short-acting insulin analogue lispro plus OADs to target PPG in T2DM. However, the glargine regimen was associated with a reduced risk of hypoglycaemia, fewer insulin injections and less self-monitoring of BG compared with lispro. Thus, the glargine regimen may improve patient treatment satisfaction and compliance compared with the lispro regimen.

Mean±SD	Glargine		Lispro	
	Baseline	*Endpoint*	*Baseline*	*Endpoint*
HbA_{1c} (%)	8.71±0.95	6.96±0.67	8.64±0.95	6.77±0.83
FBG (mmol/L)	10.3±2.0	6.2±1.5	9.9±2.7	8.0±1.9
(mg/dL)	(186±36)	(111±27)	(179±41)	(145±34)
Nocturnal BG	9.8±2.4	6.5±2.2	9.8±2.9	7.2±1.8
(mmol/L) (mg/dL)	(177±44)	(118±39)	(177±53)	(129±33)

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0146

Insulin detemir and insulin glargine: similar time-action profiles in subjects with type 2 diabetes

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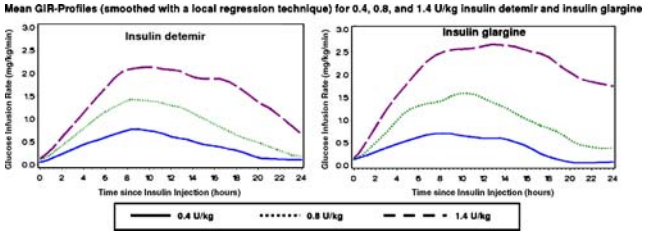
Background and Aims: This is the first head-to-head comparison of the pharmacodynamic (PD) and pharmacokinetic (PK) properties of insulin detemir (IDet) and insulin glargine (IGlar) in subjects with type 2 diabetes (T2D).

Materials and Methods: Twenty-seven insulin-treated male subjects with T2D (BMI 30.8±2.6 kg/m^2 (mean±SD), HbA1c 7.6±1.1%) were enrolled in this randomised, double-blind, parallel trial and received 0.4, 0.8, and 1.4 U/kg of IDet or IGlar under glucose clamp conditions (target blood glucose (BG) 90 mg/dL, iv insulin infusion (rate 0.3 mU/kg/min 3 h–0.5 h before dosing), clamp duration 24 h or until BG>200 mg/dL). Assessment of endogenous glucose production (EGP) and peripheral glucose uptake (PGU) were made using the isotope dilution technique (6,6–$^2H_2$–Glucose).

Results: The mean glucose infusion rate (GIR) profiles for IDet and IGlar were similar in shape/flatness (figure) and showed increasing effect with increasing dose. The dose-response relationship (log-effect versus log-dose) was similar for the insulin preparations in intercept and slope (p-values: slope=0.23, intercept=0.84). Within-subject variability was lower for IDet than for IGlar (p-values: within-subject variability <0.0001, between-subject variability=0.38). The duration of action (time from dosing to GIR <0.5 mg/kg/min) increased with rising doses for both IDet and IGlar without major difference between the two preparations for the clinically relevant doses 0.4 and 0.8 U/kg (IDet 719±512, 1007±408; IGlar: 613±443, 1162±413 min) and was >24 h in most subjects with the highest dose (1328±154 vs. 1440±0 min). IDet and IGlar led to comparable, dose-dependent suppression of EGP

(Area over EGP curve; IDet: 295±344, 610±563, 800±429 mg/kg, IGlar: 211±265, 519±427, 1034±721 mg/kg, NS for IDet vs. IGlar) and stimulation of PGU (Area under PGU curve; IDet: 393±324, 817±584, 1257±893 mg/kg, IGlar: 415±447, 721±1007, 1706±1485 mg/kg, NS for IDet vs. IGlar).



Conclusion: This first head-to-head comparison of the pharmacodynamic properties of IDet and IGlar in subjects with type 2 diabetes confirmed lower within-subject variability for IDet and showed no substantial differences in the shape of the GIR-profiles, duration of action and ability to stimulate glucose uptake and suppress endogenous glucose production.

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0147

No correlation between weight gain and number of hypoglycaemic events in patients with type 2 diabetes treated with insulin detemir as compared to NPH insulin

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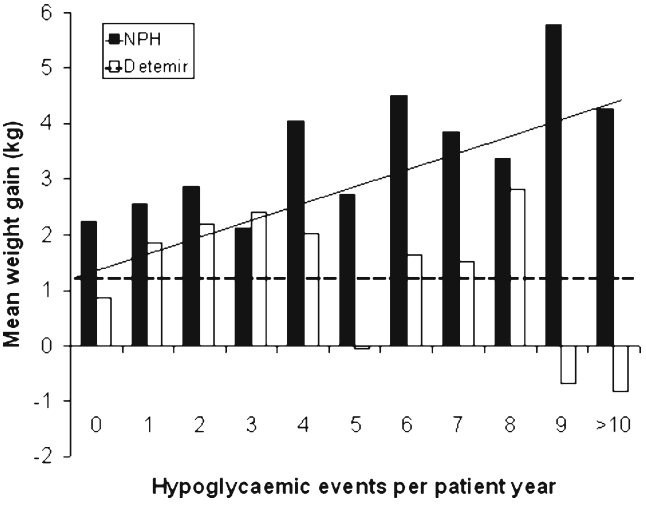
Background and Aims: Insulin detemir (detemir), a new long-acting insulin analogue, has been shown to improve glycaemic control in people with Type 1 and Type 2 diabetes when used as part of a basal-bolus regimen or as add-on to oral anti-diabetic drugs (OADs). Addition of basal insulin to OADs is a common regimen for people inadequately controlled on oral agens, but weight gain usually occurs when insulin treatment is initiated. The aim of this exploratory analysis was to investigate if any possible correlation existed between weight gain and risk of hypoglycaemia with use of detemir.

Materials and Methods: The analysis was based on a 26-week, randomised, multi-centre, open-label, parallel group trial, in which glycaemic control, risk of hypoglycaemia and weight development were compared between detemir and NPH insulin (NPH). A total of 475 insulin-naïve Type 2 diabetes patients (detemir: 237, NPH: 238) treated with one or two OADs added basal insulin morning and evening to their current oral treatment. Baseline HbA_{1c} was 8.6% (detemir) and 8.5% (NPH), respectively.

Results: Both groups achieved excellent metabolic control (HbA_{1c} detemir: 6.6%, NPH: 6.5%, NS). Weight gain was less with detemir compared to NPH (1.2 vs. 2.8 kg, $p<0.001$) and the overall risk of hypoglycaemia was 47% lower with detemir ($p<0.001$). No significant relationship between hypoglycaemia and weight gain was seen with detemir ($p=0.2$), while a statistically significant correlation was found for NPH ($p=0.003$). At an equal level of HbA_{1c}, detemir results in less weight gain than NPH. This may be caused by fewer hypoglycaemic events, which could result in less defensive snacking with detemir.

Conclusion: The results of this analysis contribute to our understanding of some of the underlying mechanisms of insulin associated weight differences between detemir and NPH.



Supported by Novo Nordisk A/S

0148

Insulin glulisine: faster onset of absorption and action than insulin lispro in lean to obese subjects

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Background and Aims: This randomized, double-blind, cross-over study compared the pharmacodynamic (PD) and pharmacokinetic (PK) properties of the rapid-acting insulin analogues insulin glulisine (GLU) and insulin lispro (LIS) in lean to obese subjects.

Materials and Methods: Eighty healthy subjects were stratified into 4 body mass index (BMI) classes (<25, 25≥30, 30>35 and≥35 kg/m^2) and received single injections of GLU and LIS (0.2 U/kg and 0.4 U/kg) in a randomized order on 4 study days under euglycaemic glucose clamp conditions. Glucose infusion rate (GIR) and insulin (INS) concentration were assessed for 10 hours post-dose. Areas under the curve (AUC) from individual INS and GIR profiles were calculated from untransformed data with the trapezoidal rule. All PD and PK data (after a natural log-transformation) were analysed using an analysis of variance model to allow the estimation of least square means and to test differences between treatments.

Results: Early metabolic action (GIR-AUC$_{0–1h}$) was significantly greater with GLU than with LIS in the total population and in a number of BMI groups ($p<0.05$, see Table) while the total metabolic effect was not significantly different between treatment groups. Correspondingly, onset of action (GIR-$t_{10\%}$) was faster with GLU than with LIS. This is in agreement with a consistently faster early INS exposure (INS-$t_{10\%}$) with GLU compared with LIS at either dose.

Conclusion: These findings in lean to obese subjects support previous observations of a slightly faster BMI-independent onset of action of GLU in obese to morbidly obese subjects. Because of its faster onset of action, GLU may offer improved postprandial glucose control.

	BMI (kg/m^2)	0.2 U/kg GLU – LIS	0.4 U/kg GLU – LIS
GIR-AUC$_{0–1h}$ (mg/kg)	**Total**	**19.1 (6.6;31.7)***	**45.6 (33.1;58.2)***
	<25	18.2 (−6.8;43.3)	78.6 (53.5;103.7)*
	25≥30	28.9 (3.8;54.0)*	33.0 (7.9;58.1)*
	30>35	10.6 (−14.5;35.7)	54.0 (28.8;79.0)*
	≥35	18.7 (−6.4;43.8)	17.1 (−8.0;42.2)
GIR-$t_{10\%}$ (min)	**Total**	**−4.5 (−8.0;−1.0)***	**−4.0 (−6.5;−1.0)***
	<25	−3.5 (−9.5;2.0)	−6.0 (−9.0;−2.5)*
	25≥30	−5.0 (−13.0;0.0)*	−4.3 (−10.0;1.5)
	30>35	−2.5 (−11.5;9.5)	−5.3 (−13.0;1.0)
	≥35	−5.0 (−18.0;9.0)	1.5 (−6.5;10.0)
INS-$t_{10\%}$ (min)	**Total**	**−5.4 (−7.0;−3.8)***	**−5.0 (−6.8;−3.3)***
	<25	−4.3 (−7.0;−1.3)*	−6.4 (−8.5;−3.8)*
	25≥30	−4.6 (−10.9;−2.6)*	−8.8 (−12.3;−5.2)*
	30>35	−5.4 (−8.7;−1.3)*	−3.0 (−6.9;−0.2)*
	≥35	−7.0 (−10.6;−2.7)*	−0.9 (−6.1;4.0)

Values are point estimates with 95% confidence limits of differences between treatments; *p<0.05 versus corresponding dose and BMI group for LIS

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0149

Sexual activity in diabetic patients treated by continuous subcutaneous insulin therapy

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Background and Aims: Concerns about interference with sexual activity could be an obstacle to initiating pump therapy in type 1 diabetic patients. The aim of this study was to evaluate the level of sexual activity reported by diabetic patients treated with insulin pump therapy (CSII).

Materials and Methods: Between July 2005 and December 2005, patients filled out a questionnaire concerning their demographic data, diabetes history, pump treatment history, metabolic control, the inconvenient/convenient from pump and catheter (via visual analogic scales) and information on sexual activity.

Results: 336 diabetic patients treated with CSII since 4.2+/−5.9 yrs, age 43+/−15.9 yrs, women 53%, diabetes duration 19+/−11 yrs, single 20%, were included in a cohort by 42 diabetologist in 15 hospital center or private diabetologic center. Pump indications were : bad metabolic control (HbA1c>8%) :42%, unstability:34%, pregnancy:13%, injections intolerance:6%, life style :2%, insulin resistance :3%. HbA1c was 7.8+/−1.26%, with 2.45+/−2.27 mild hypoG/week. Complications: retinopathy: 45.2%, nephropathy: 17.8%, neuropathy: 31.8%, macroangiopathy: 9.9%. Sexual activity frequency was: never: 29.9%, <1/month: 12.3%,>1/month and <1/week: 18.2%, >1/week: 39.6%. These frequencies were correlated with age ($p<0.0001$), co-habitation ($p<0.01$), good metabolic control ($p<0.05$), absence of complications (no nephropathy ($p<0.05$), no neuropathy ($p<0.05$), and no macroangiopathy ($p<0.01$)), but not with “dislike/like” pump or catheter. Multivariate analysis showed that only age and co-habitation were correlated with sexual activity frequency ($p<0.0001$ and 0.0003 respectively) but not with diabetes duration or complications. To the question: “Does the pump

have an influence on your sexual activity?" the answer was: no: 89.4%, yes: 10.6%. In multivariate analysis, this influence was correlated with HbA1c ($p<0.05$), the fact to report a discomfort with the pump ($p<0.05$), the number of mild hypoglycaemia ($p<0.01$). Finally, to the question: "Do you disconnect the catheter during sexual activity?" the answer was: never: 22.3%, rarely: 11.6%, sometime: 18.2%, always: 47.9%. Multivariate analysis showed that men and younger people disconnect the pump more often ($p<0.05$). This was also the case for patient with bad metabolic control ($p<0.05$) and those who reported inconvenient by the pump. This disconnexion was less frequent in patient with high pump duration ($p<0.01$).

Conclusion: Sexual activity frequency seems not affected by pump therapy or diabetes but is correlated with age and co-habitation. The use of the pump (i.e. disconnection) during sexual activity is different according the gender, age and pump duration.

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0150

Ten year-experience with implanted insulin pumps documents safe and effective control in type 1 diabetic patients with initial poor prognosis

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Background and Aims: Early studies with implanted insulin pumps (IP) from 1989 to 1993 proved treatment feasibility, safety and effectiveness on glucose control in type 1 diabetes mellitus. After poor physical stability of infused U400 insulin, that occurred in 1993–1994 and resulted in recurrent underdelivery incidents, has been improved, the EVADIAC group resumed implantations of IP from 1995 to assess long-term safety and effectiveness of this therapy. The goal of the present study was to investigate the benefit- risk ratio of IP therapy on a long-term basis.

Materials and Methods: Current cumulated data concern 137 type 1 diabetic patients (58 males, 79 females) who started with IP therapy between 1995 and 2005. At implantation time (T0), mean age was 42.3 (range:19–63) yrs and diabetes duration 22.3 (2–52) yrs. Status of diabetic complications included neuropathy in 30.4% of patients, nephropathy in 23.4%, retinopathy in 60.4%, cardiovascular disease in 15%. Prior to implantation, insulin treatment regimen was MDI in 17.2% of patients and CSII in 82.8%. Motivations for pump implantation included: poor blood glucose stability (43.1%), recurrent hypoglycaemia (either severe or more than four non severe per week) (33.1%), poor metabolic control based upon HbA1c levels (10%), SC insulin resistance (4.6%), others (9.2%).

Results: Reported mean duration of treatment with IP was 46.4 months (6–124) with most of the patients currently continuing this therapy. HbA1c levels significantly decreased from T0 to T1 (one year later) (in 128 patients) i.e. 7.91 to 7.69% ($p<0.003$), T0 to T2 (two years later) (in 102 patients) i.e. 7.92 to 7.69% ($p=0.023$), T0 to T4 (in 46 patients) i.e. 8 to 7.69% ($p=0.04$) and from T0 to T5 (in 34 patients) i.e. 8 to 7.6% ($p=0.03$). Meanwhile, no significant variation of body weight and complication status assessed on a yearly basis occurred. No correlation was identified between body weight and HbA1c levels. Seventeen premature pump removals were motivated by blocked pumps (n=5), premature low battery power (n=4), pump-pocket complications (n=5), recurrent catheter encapsulations (n=3).

Conclusions: This unique and large-scale experience supports a positive benefit upon risk ratio for IP therapy in poorly controlled type 1 diabetes mellitus in spite of an intensive SC insulin treatment regimen, mostly associated with advanced complications. From these data, IP should deserve a renewed interest as a mean to improve metabolic status and impaired prognosis of these patients, when more widely available.

OP 26

Wound healing — diabetic foot

0151

Novel PKC based therapeutic overcoming diabetes related wound healing impairment

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Background and Aims: Diabetes involves skin related complications including chronic untreatable wounds which lead, in many cases, to lower extremity amputations. Protein kinase C (PKC), a family of serine-threonine kinases is known to be involved in skin physiology and diabetes pathology as well as in regulation of glucose homeostasis. In this study we introduce an innovative PKC based formulation for the treatment of diabetes ulcers, composed of a novel PKC activating peptide and a classical PKC inhibiting peptide (HO/03/03).
Materials and Methods: Wound healing studies were set in two model systems: STZ (175 mg/kg) injected C57BL mice which were sustained until blood glucose levels were>450 mg/dl for at least 2 days (diabetes type 1 model) and Psammomys Obesus (diabetes type 2 model); biopsies were collected 7–9 days post wounding. Morphological analysis of healing progression was performed during the experiment followed by a novel quantitative immunohistochemical assessment strategy carried out by utilizing specific epidermal and dermal markers: keratin 14 (K14) staining (epidermal regeneration), keratin 1 (K1) (epidermal differentiation), keratin 6 (K6) (epidermal migration), proliferating cell nuclear antigen (PCNA) (cell proliferation and granulation tissue formation) and Masson trichrome staining for collagen fiber distribution. Dermal contraction and remodeling were assessed by relative wound size and hair follicles regeneration. Organization of the hypodermis and severity of inflammation were assessed utilizing hematoxylin–eosin (H&E) and myeloperoxidase.
Results: Our results demonstrate a uniform wound healing impairment in both diabetic animal models including all parameters tested: epidermal closure 67% vs. 100% in diabetics and non diabetic wounds respectively; a complete absence of hypodermis layer in diabetics vs. 100% in controls; severe inflammation in 83% vs. 33%; granulation tissue formation 50% vs. 100% in controls. In addition, abnormal angiogenesis in the wound gap was distinctive of diabetic wounds (83% vs. 0). These results clearly demonstrate that the diabetic state is consistent with wound healing impairment. Topical treatment with HO/03/03 dramatically induced wound healing and accelerated critical healing parameters when compared to control diabetes non treated wounds including: epidermal closure (71% vs. 17%), epidermal differentiation (33% vs. 0%) and dermal contraction (28% vs. 0%). In addition, HO/03/03 significantly reduced the inflammatory response characteristic of impaired diabetic wounds.Further examination of non healing wounds in larger animal models, such as dogs and horses, showed that treatment with HO/03/03, accelerated wound healing similarly to diabetic animal wounds. A clinical trial protocol using this formulation for the treatment of diabetic ulcers was approved by the Israeli Ministry of Health. Preliminary human case studies of chronic non healing wounds revealed positive results in facilitating wound closure when treated with HO/03/03.
Conclusion: These results demonstrate that specific PKC modulation is imperative to wound healing processes and can overcome the pathology of impaired wound healing as it appears in diabetes. Moreover, we demonstrated the therapeutic ability of a novel PKC based formulation (HO/03/03) in impaired wound healing in various models.

0152

Negative pressure wound therapy in complex diabetic foot wounds: costs, resource utilisation and outcomes

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Background and Aims: To report on health economic and resource utilization aspects of negative pressure wound therapy (NPWT) compared to standard wound therapy (SWT) based on results of a randomized clinical trial.
Methods: 162 patients were enrolled into a sixteen-week, eighteen-center randomized clinical trial. Inclusion criteria consisted of partial foot amputation wounds up to the transmetatarsal level and evidence of adequate perfusion. Patients randomized to NPWT (n=77) received therapy with dressing changes every 48 hours. Control patients (n=85) received SWT according to consensus guidelines. NPWT was delivered through the Vacuum Assisted Closure (V.A.C.) Therapy System. Wounds were treated until healing or completion of the active treatment period.
Results: NPWT was superior to SWT in both proportion ($p=0.04$) and rate ($p=0.005$) of healing. The median time to healing in the NPWT group was 8 weeks compared to 11 weeks for SWT. More reamputation (10.6% vs. 2.6% and all high level amputations (5.9% vs. 0%) occurred in the SWT group compared to the control group ($p=0.06$). Total skilled nursing hours dedicated to the wound was 3-fold higher in the SWT group (86.5+/− 76.6 vs. 27.6+/− 16.1, $p<0.01$). Independent of healing status, the number of dressing changes per 24-hour period was lower in the NPWT group (0.5+/− 0.2 vs. 1.4+/− 0.6 , $p<0.01$) compared to SWT. 61% fewer dressing changes (603 vs. 1555) were performed by physicians in the NPWT group compared to SWT. This trend was consistent for other health care providers and family members ($p=0.03$). Estimated hospital-

ization and procedure-related costs for reamputation alone (excluding prostheses, disability and rehabilitation) were lower in NPWT (\$79,913 vs. \$362,840 USD).
Conclusion: Treatment with NPWT resulted in a higher proportion of wounds that healed at an improved rate, while at the same time resulting in less resource utilization than SWT. Because of the reduced number of interactions required with the wound, weekly reductions in utilization and direct cost may be independent of healing status.
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0153

Thrombin-activated platelets enhance healing of ulcerative skin lesion by inducing IGF-1 release by skin fibroblasts

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Background and Aims: Diabetic foot is a common and hard to treat complication of diabetes accompanied by a strong impairment of life quality. Platelet components have found successful clinical utilization to initiate or to accelerate tissue repair in this pathology. However, the molecular pathways by which platelet factors contribute to tissue regeneration have not been fully elucidated. We have studied the effect of thrombin-activated platelets (TAPs) on cell growth *in vivo* and in cultured cell systems.
Materials and Methods: Shoulder skin fibroblasts were obtained by punch biopsy and were grown at 37 C in DMEM supplemented with 10% fetal calf serum in a 5% CO_2-95% air humidified atmosphere. Cultures were used for experimental procedures between the 8th and 15th passage. Platelets'rich plasma (PRP) was obtained from healthy donor volunteers (for *in vitro* experiments) and from diabetic patients undergoing autologous TAPs treatment for ulcers of the lower extremities. For TAPs preparations, thrombin (1:10 vol/vol) and calcium gluconate (1:10 vol/vol) were added to PRP for 5 min at room temperature. For the treatment of the patients, the volumes were dictated by the size of the lesions and the applications were repeated twice a week. For *in vitro* experiments, 1 cm^2 aliquots of PG were applied to cells plated in 100 mm diameter dishes.
Results: Application of TAPs to ulcerative skin lesions of diabetic patients induced local activation of ERK1/2 and Akt/PKB. Moreover, when applied to cultured human skin fibroblasts, obtained both from healthy donors and diabetic patients, TAPs promoted cell growth, DNA synthesis and activated PDGF and IGF-1 receptor tyrosine kinases. PDGF was released by TAPs and rapidly achieved a plateau. At variance, the release of IGF-1 was mainly provided by the TAPs-stimulated fibroblasts and progressively increased up to 48 h. The PDGF-R blocker Ag1296 reduced the activation of Akt/PKB and, at a lesser extent, of ERK1/2. Conversely, inhibition of IGF-1 signaling by Ag1024 and expression of a dominant negative IGF-1R mutant selectively reduced the stimulation of ERK1/2 by TAPs and fibroblast-released factors, with minor changes of Akt/PKB activity.
Conclusion: Here we show that TAPs represent an important source of growth factors to induce the proliferation of human fibroblasts, *in vivo* as well as in cultured systems. Platelet factors promote fibroblast growth by acutely activating Akt/PKB and ERK1/2. Sustained activation of ERK1/2, however, requires autocrine production of IGF-1 by TAPs-stimulated fibroblasts.

0154

Use of foam dressing for lower-extremity necrotising fasciitis in type 2 diabetic patients

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Background and Aims: Extensive necrotising fasciitis requiring major debridement belongs to the most severe diabetic foot complications. Foam dressings retain moisture, absorb exudates, facilitate removal of slough, and thereby promote wound healing. The aim of this study was to investigate whether foam dressings are superior to conventional dressings in type 2 diabetic patients with lower-extremity necrotising fasciitis.
Materials and Methods: This study included 48 type 2 patients (28 men, 20 women) with a mean age of 65±10 years and a mean diabetes duration of 9±5 years. The study was conducted between 1st January and 31st July 2005. All patients suffered from lower-extremity necrotising fasciitis and had already undergone debridement. Patients were randomly assigned to the study group and the control group. In the study group (N=24), foam of 10 mm×10 cm×30 cm and shore hardness of 10 was used, without topical antibiotics or desloughing ointments. In the control group (N=24), patients were dressed with conventional techniques, using antibiotic and hydrocolloid ointments. Dressings were changed on a daily basis and bedside debridement was performed as required in both groups. Patients were offloaded on the operated limb in

both groups. When sufficient granulation tissue was evident and the ulceration exceeded 5 cm in diameter, split skin grafting (s.s.g) was employed. Time to heal was regarded as the number of days from 1st debridement until s.s.g. In patients who were not skin-grafted, time to heal was taken as the number of days from 1st debridement until complete healing or until 31st July 2005.

Results: Successful granulation to enable s.s.g was accomplished in 19 patients (79.2%) in the study group vs. 11 patients (45.8%) in the control group (p=0.037), denoting better granulation in the study group. Failure to heal completely was observed in 7 patients (29.2%) in the control group, while all patients in the study group healed completely by the end of the study (p=0.009). The mean time to heal was 22.5+15.4 days in the foam group, while it was 48.6+21.0 days in the control group (p<0.0001).

Conclusions: Application of foam dressing is associated with higher healing rates and a significantly shorter time to heal as compared to treatment with conventional dressings in type 2 diabetic patients with lower-extremity necrotising fasciitis. These encouraging results warrant further investigation to address the issue of the potential reduction in hospital stay, treatment costs and morbidity.

0155

Accelerated cutaneous wound healing of streptozotocin diabetic mice via non-viral VEGF^{165} gene therapy using biocompatible super-coiled minicircle plasmid

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Background and Aims: Chronic un-healing foot wound is a serious problem in the complicated diabetic patients. To accelerate the healing of diabetic cutaneous wounds, various kinds of growth factors have been employed with the limited success. The short half life of the administered growth factors in the hostile wound beds might have limited wide-spread clinical usage. To overcome this, growth factor gene therapy might be an attractive alternative rather than the direct application of factors onto the wound beds. However, possible biological risks of foreign DNA integration into the chromosome and the immunologic side effects of viral gene carrier have made it very difficult to be used in the actual human treatment. To develop the more safe and effective non-viral growth factor gene therapy techniques, we performed this study.

Materials and Methods: We cloned super-coiled minicircle plasmid containing human VEGF^{165} cDNA devoid of hazardous bacterially originated sequences including CpG motifs which could stimulate the host immune responses. We injected mincircle pMINI-beta-VEGF165 via non-viral gene delivery techniques into the margin of cutaneous wound of streptozotocin diabetic C57BL/6J mice.

Results: Administration of pMINI-beta-VEGF^{165} showed much accelerated wound healing as compared with the control groups without the any adverse events. Increased neovascularizations in the wound were confirmed by the quantitative and qualitative laser Doppler flowmetry and the immunohistochemical staining of the various vascular markers. The VEGF^{165} expression decreased again to the basal level shortly after the healing of cutaneous wounds.

Conclusion: These data might suggest that the non-viral VEGF165 minicircle plasmid gene therapy can be effectively used without the harmful side effects of viral gene therapy for the treatment of chronic un-healing cutaneous wounds of diabetic patients.

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0156

Long-term effects of intranasal calcitonin on healing times and recurrence of acute Charcot foot: a randomized controlled trial

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Background and Aims: Charcot foot represents a late complication of diabetes and can lead to progressive and worsening disruption of the bone architecture of the foot. The aim of this study was to evaluate the effectiveness of intra-nasal calcitonin on clinical features (healing times and recurrence of acute Charcot foot attack) in a randomized controlled trial.

Materials and Methods: Thirty-six patients with acute Charcot foot treated in our foot clinic during a 16-month period were randomized to receive intranasal calcitonin 200 IU/day together with a calcium supplementation 1000 mg/day [study group (n=18)] or on calcium supplementation of 1000 mg/day in monotherapy [control group (n=18)]. All patients also had standard treatment of the Charcot foot, including off-loading by removable contact cast or cast walkers, with no significant difference between groups in the type of used off-loading device. All subjects were instructed to bear weight on their casted extremity using assistive devices (crutches). Healing was defined as

the cessation of off-loading. Until March 2006, 31 (sixteen in the study group and fifteen patients in controls), who were primary healed and finished a one-year follow-up period after primary healing, were assessed in our preliminary study. Healing time was defined as the number of days from the initial off-loading device application until the cessation of the casting. The decision of definitive off-loading cessation was made on the basis of clinical signs (foot without oedema and redness with skin temperature difference <2°C during 6 weeks period of temporary intermittent off-loading) and a favourable radiological and isotopic examination of affected foot. The recurrence was defined as new signs of acute Charcot foot requiring new intensive off-loading and it was assessed during one- year period since cessation of casting.

Results: The mid-foot was the most commonly affected site [13 (81%) in the study group, 13 (86%) in controls; NS]. There were a significant shorter healing times in the study group in comparison with controls [mean 159.8 days (95% CI 137.6–182.1) vs. 195.2 days (95% CI 166.8–223.7); $p<0.05$]. Three in the study group (18.7%) and six (40%) subjects in controls had acute recurrences of Charcot foot within the first year of follow-up after the initial casting period, but no significant difference was seen between groups. Seven patients in each group were ulcerated (43.7% vs. 46.6%; NS) and two patients underwent minor amputation in both groups during the study period (12.5% vs. 13.3%; NS), no major amputation was required.

Conclusion: This study suggests that patients with acute Charcot foot may have a clinically apparent benefit from the calcitonin treatment, which shortens healing times of the Charcot foot. The tendency of the lowering the recurrence rate of acute Charcot foot attack is promising, but a longer follow-up period is necessary.

OP 27

Genetics of type 1 diabetes

0157

Genetic interaction in type 1 diabetes susceptibility supported by data mining and protein interaction analyses

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Background and Aims: Complex traits like type 1 diabetes (T1D) are influenced by multiple genes presumed to interact with each other to confer disease susceptibility and/or protection. The aim of the present study was to use analytical methods of data mining and decision trees to evaluate genetic interaction in T1D pathogenesis. For evaluation of functionality of observed genetic interactions, protein interaction networks were generated.

Material and Methods: T1D genome scan data (IBD-status) on 1321 affected sib pairs, obtained via the Type 1 Diabetes Genetics Consortium, was used. Decision tree learning (SAS Enterprise Miner) was used to detect combinations (rules) of microsatellite markers splitting data into cleaner subgroups of T1D vs. Non-T1D. Proteins encoded by 10 Mb on each site of microsatellite markers included in the rules were used as input proteins, and queried by a newly developed protein interaction network analysis scheme, for interactions at the protein level. Resulting protein interaction networks were visualized by Cytoscape v.2.0.

Results: Evidence for genetic interaction between several loci was observed. Rules of two markers providing more information than any of the markers themselves were obtained (P=<0.001 to 0.04). For further evaluation of these predicted genetic interactions we queried the loci for physical interactions with each other. By generation of protein interaction networks, we demonstrated evidence for interactions at the protein level for several of the most significant genetic interactions predicted by data mining studies, e.g. between the HLA-region and a region on chromosome 19 (*TNFA - D19S591*), as well as between HLA and chromosome 21 (*TNFA - D21S270*), and between regions on chromosome 17 and 21 (*D17S798 - D21S167*). The molecular function and biological processes of several of the proteins in the high-confidence networks supports oxidative stress, signal transduction, regulation of transcription and apoptosis to be important in T1D pathogenesis.

Conclusion: Implication for functionality was obtained for several of the observed genetic interactions conferring susceptibility to T1D. This may be the most extended suggestion of genetic epistasis in a multifactorial disease as T1D, strengthened significantly by the functional evidence for the interactions. This support the concept of genes encoding proteins, which function within complex networks to carry out specific biological processes, and supports that genetic interaction is important in T1D pathogenesis.

The Type 1 Diabetes Genetics Consortium (www.t1dgc.org) kindly provided access to their genome scan data.

0158

Differential expression of *HLA-DQA1* and -*DQB1* alleles associated with susceptibility to and protection from autoimmune type 1 diabetes

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Background and Aims: The risk of developing type 1 diabetes is influenced by HLA-DQ alleles; *DQA1*0301*, *DQA1*0501*, *DQB1*0201* and *DQB1*0302* predispose to the disease while *DQA1*0102* and *DQB1*0602* confer protection. This effect may be related to the ability of the encoded molecules to present diabetogenic antigens to autoreactive T cells. The three-dimensional structure of the DQ molecules is an important determinant of this function, but the activation of an autoimmune response may also be influenced by the density of these molecules on the surface of antigen presenting cells. Disease risk may therefore be determined by the relative levels of expression of predisposing and protective/neutral alleles. To explore this possibility, we investigated the relative levels of mRNA transcripts from pairs of DQA1 and DQB1 alleles in peripheral blood lymphocytes (PBLs) from DQ-heterozygous individuals with and without type 1 diabetes. We also assessed the promoter activity of the alleles associated with the disease. Our aim was to determine whether a) different alleles are expressed at different levels, b) these differences are attributable to differences in promoter activity, c) the relative level of expression of an allele is related to its influence on disease risk and d) the ratio of expression of protective and predisposing alleles correlates with disease risk.
Materials and Methods: The relative abundance of mRNA transcripts from pairs of DQA1 and DQB1 alleles was determined in 59 DQ-heterozygous individuals (29 with type 1 diabetes and 30 healthy controls) using RT-PCR followed by allele-specific restriction digestion. The proximal promoter and polymorphic upstream regulatory regions of the predisposing and protective DQ alleles were cloned into the pGL3 Basic vector. The constructs were introduced into three DQ homozygous B lymphocyte cell lines using electroporation and promoter activity was measured using the Dual-Glo luciferase assay.
Results: The *DQA1*0301* and *DQB1*06* alleles were generally over-expressed at the mRNA level compared to other alleles. In contrast, mRNA for the *DQB1*02* allele was generally less abundant than other DQB1 transcripts There was no consistent pattern of expression for *DQA1*0102* and *DQA1*0501*. The ratio of expression of predisposing/protective alleles did not differ between the diabetic and control subjects. The results of the promoter study were concordant with those from the mRNA analysis. The *DQA1*0301* promoter showed higher activity compared with *DQA1*0102* and *DQA1*0501*; no consistent pattern was observed for the latter two alleles. The *DQB1*06*02 promoter showed greater activity compared with *DQB1*0201* and *DQB1*0302*.
Conclusion: This is the first study to demonstrate differential allelic expression at both the DQA1 and DQB1 loci in PBLs. Our data suggest that the relative abundance of mRNA transcripts for different alleles may be partly determined by differences in promoter activity. No simple correlation was found between the level of expression of specific DQ alleles and their influence on the risk of diabetes. The unbalanced expression of DQ alleles may influence the stoichiometry of the DQ αβ heterodimers expressed on the surface of antigen presenting cells. This could have important implications for the pathogenesis of autoimmune disease and requires further study at the protein level.
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0159

Major histocompatibility complex, class II, transactivator , MHC2TA, is associated with type 1 diabetes susceptibility

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Background and Aims: The major histocompatibility complex (MHCII) is thought to be the major susceptibility factor for type 1 diabetes (T1DM). Other non-HLA genetic factors are also shown to contribute to T1DM susceptibility. The MHC2TA (16p13) gene codes for the non DNA binding transcriptional co-activator CIITA. This protein is transcribed from four different MHC2TA promoters (PI-PIV).The CIITA protein is crucial for MHC II transcription and it is therefore thought that the MHC2TA gene is the main controlling factor of MHC II expression. The rs3087456 marker located in the PIII region of the MHC2TA gene, has been reported to be involved in the susceptibility of Multiple Sclerosis (MS), Rheumatoid Arthritis (RA) and Myocardial Infarction. We have previously observed linkage in the region of MHC2TA to

T1DM. Thus the aim of our study was to test the hypothesis that MHC2TA is a T1DM susceptibility gene.
Material and Methods: Genotyping was carried out using the DASH method and the Taqman system. Genotypes from 28 Single Nucleotide Polymorphisms (SNP) in 373 unrelated Caucasian controls were used to define haplotype blocks and tagging SNPs in the HapBlock program. In total 20 of these SNPs were genotyped in a Swedish case-control study (586 T1DM patients and 836 controls). The age at onset of the patients varied between 15 and 34 years of age. Test of association were calculated using the cocaphase command in the Unphased computer program. Logistic regression analysis was performed using the proc logistic command in the SAS package.
Results: The HapBlock program revealed 5 blocks in the MHC2TA gene extending from before PI to the 3́UTR region. The blocks were tagged by 1 to 3 SNPs each, comprising a total of 12 SNPs. These SNPs were analysed in the Swedish case-control study to show that SNPs in the first two haplotype blocks in MHC2TA were associated with T1DM ($p<0.006$ and $p<0.04$ respectively). An additional 8 SNPs were typed in these two blocks (see figure 1). The rs3087456 marker, in PIII, which was associated to MS, RA and MI was also associated with T1DM ($p<0.04$), however a stronger association is observed for rs11074932, between PI and PIII, $p<0.003$. The association of MHC2TA remained significant in a logistic regression analysis including HLA DRB1, DQA1 and DQB1 as well as INS VNTR. The presence of T at rs11074932 was associated with T1DM (OR=2.48, 95%CI 1.25–4.92, p=0.003).
Conclusion: We conclude that the rs11074932 SNP located between PI and PIII is a significant susceptibility marker for T1DM. However, since no markers located in PI could be typed and due to the close proximity of rs11074932 to PI it is suggested that sequencing of PI should be done in order to fully understand which marker "truly" is involved in the susceptibility of T1DM. Taken together these data, we propose that the MHC2TA gene contributes to the susceptibility of T1DM.
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0160

Association of *FOXP3* polymorphisms with GAD65 autoantibodies in type 1 diabetes

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Background and Aims: Although increasing efforts have been made to identify susceptibility genes, only a few candidate genes besides the MHC have clearly been associated with type 1 diabetes (T1D) development. The human *FOXP3* is a forkhead transcription factor, encoded by a gene on chromosome Xp11.23, which is important for the development and function of regulatory T cells. The aim of this study was to define if variants in the *FOXP3* gene are associated with T1D development.
Materials and Methods: We screened single nucleotide polymorphisms (SNPs) within the regulatory T cell specific gene *FOXP3* among 803 T1D patients and compared the allele frequencies with 208 healthy individuals. Importantly, we also correlated the SNPs with various clinical parameters in the T1D patient group, including levels of GAD65 autoantibody (GADA), HbA1c, fasting plasma glucose, fasting plasma C-peptide, body mass index (BMI) and HLA genotypes.
Results: Three of four SNPs were in linkage disequilibrium (LD), and one of these was thoroughly analyzed. Male T1D patients with the minor A allele of *FOXP3* rs 2232365 (2365) displayed lower frequencies of GADA (p=0.004) and lower GADA titers (p=0.003) compared to those with the G allele. When only male T1D patients with short disease duration (0 to 3 years) were considered, patients with the G allele had higher frequencies of undetectable C-peptide (30.3%) than those with the A allele (4.2%; p=0.02). When patients were stratified for HLA genotypes a significant difference in the frequency of GADA positive individuals and GADA titers in the male T1D group with risk HLA-DQB1 genotypes was observed. Logistic regression analyses among all T1D males demonstrated that GADA were associated with shorter disease duration, older age, older age at onset of diabetes, HLA-DQB1 risk genotypes and with the *FOXP3* 2365 G allele (p=0.0001).
Conclusion: The minor A allele in the *FOXP3* 2365 SNP seems to be a protective allele in T1D development and therefore this novel finding suggests a role of *FOXP3* in the pathogenesis of T1D.
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0161

TIM-3 and CTLA-4 polymorphisms in German type 1 diabetes families: two genes associated with T-lymphocyte regulation

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Background: The balance between Th1 and Th2 cells is critical in the pathogenesis of type 1 diabetes. It has been previously reported that the family of T-cell immunoglobulin domain and mucin domain (*TIM*) proteins are expressed on the T cells. *TIM-3* is present in Th1 cells and appears to play an essential role in Th1-mediated immune responses and in macrophage activation. While the cytotoxic T lymphocyte antigen-4 (CTLA-4) is a negative regulator of activated T-cells, TIM-3 downregulates the T-lymphocyte response at the effector level. Furthermore TIM-3 deficient mice develop a more severe form of experimental autoimmune diabetes. Therefore we investigated the role of *TIM-3* gene polymorphisms in German type 1 diabetes families.
Materials and Methods: German type 1 diabetes families [191, (573 subjects)] were genotyped for the rs10515746 and rs1036199 polymorphisms using real time PCR, while for the rs10053538 by restriction fragment length polymorphism. Genotype-, haplotype-frequencies and linkage disequilibrium (LD) were estimated using Unphased version 2.403. A corrected *P* (*P*c)<0.05 was considered significant.
Results: No differences were observed in the transmission and not transmission frequencies of the *TIM-3* polymorphisms ($P_{rs10515746}$=0.6548, $P_{rs1036199}$=0.7114, $P_{rs10053538}$=0.2214, respectively). Allele frequencies range from 5 to 20%.
We correlated the two CTLA-4 polymorphisms with the strongest type 1 diabetes association (CT_60 and MH30) with TIM-3. In the presence of TIM-3-rs10053538C the relative risk to type 1 diabetes - stratified for the polymorphisms CTLA-4 MH30 or CT_60 - is higher than if these CTLA-4 SNPs were considered alone: odds ratios rise from 1.83 to 1.93 and from 1.57 to 1.64, respectively, but this difference is not significant. Thus, the allele combinations rs10053538C-MH30G (*P*=0.0001) and rs10053538C-CT_60G (*P*=0.0011) were more frequently transmitted, while the allele combinations rs10053538C-MH30C and rs10053538C-CT_60A were less often transmitted to offspring (*P*=0.0002 and *P*=0.0041).
Conclusion: A combined transmission of TIM-3 and CTLA-4 variants on risk haplotypes enhances the genetic susceptibility to type 1 diabetes but the TIM-3 contribution is marginal.

0162

Temporal trends in HLA genotype distribution in 1986–2005 suggest altered risk for type 1 diabetes

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Background: It has been suggested that the frequency of certain HLA genotypes among new onset type 1 diabetes patients may show a temporal change. The relative risk of certain HLA genotypes are gaining while others are losing in risk. Such changes may influence the triggering events, the age at clinical onset and may perhaps explain the trend that children tend to develop the disease at a younger age.
Aims: Our aims were to compare the frequency of HLA genotypes in new onset type 1 diabetes patients diagnosed at 1–18 years of age in 1986–1987 (n=430), 1996–2000 (n=342) and 2003–2005 (n=171). We tested the hypothesis that the distribution of the HLA genotype frequencies changes over time.
Methods: Consecutively diagnosed Swedish type 1 diabetes patients were typed for HLA using PCR amplification and allele–specific probes for DQ A1* and B1* alleles. Buffy coats or Dried Blood Spots were used as the source of DNA. The case–control study of all patients developing type 1 diabetes in 1986–87 was the baseline reference study available at http://stat-db.stat.sfu.ca/magenta/. The 1996–2000 cohort were ascertained in Ská¥ne and the 2003–2005 group from Stockholm.
Results: The most common T1D HLA genotype DQA1*-B1*: 0501–0201/0301–0302 changed from 36% (153/430) in 1986–87 to 37% (127/342) in 1996–2000 and to 19% (33/171) in 2003–2005. The decrease in the high-risk heterozygous genotype may be mirrored by an increase in the homozygous 0301–0302/0301–0302 genotype: 8% in 1986–87, 10% in 1996–2000 and 12% in 2003–2005. There was also an increase in the frequency of the 0301–0302/0102–0604 genotype: 2% in 1986–87; 2% in 1996–2000 and 4% in 2003–2005. The 0501–0201/0501–0201 genotype also increased: 2% in 1986–87; 7% in 1996–2000 and 5% in 2003–2005. All changes over time were significant (p<0.05).
Conclusion: This study in Swedish type 1 diabetes patients diagnosed at 1–18 years of age support the notion that there is a temporal change in HLA risk.
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OP 28

Nephropathy – experimental

0163

Podocyte inducible angiopoietin-2 overexpression results in albuminuria and glomerulosclerosis in transgenic mice

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Background and Aims: The development of nephropathy in diabetes is associated with glomerular upregulation of angiopoietin-2 (Ang2), a critical growth factor implicated in angiogenesis and vascular integrity. The specific role of this growth factor in renal dysfunction and pathology is unknown.
Materials and Methods: We generated transgenic animals with glomerular specific overexpression of Ang2 and β-galactosidase cDNA using the *Tet-On* regulatable inducible system. We utilised the podocyte-specific promoter podocin driving the expression of the reverse tetracycline transactivator. To activate the transgene, Doxycycline (2 mg/mL) was administered with water. We studied the following groups of mice for up to 10 weeks: **a**) transgenic (TG) with doxycycline (DOX), **b**) TG without DOX, **c**) controls (C) with DOX and **d**) C without DOX. Kidney cortex was collected and prepared for subsequent analysis for β-galactosidase staining, western immunoblotting, immunohistochemistry, and electron microscopy studies. 24-hour albuminuria was determined with ELISA.
Results: Podocyte positive β-galactosidase staining was present only in TG-DOX treated animals, indicating absence of leaky transgene activity. Western immunoblotting and immunogold studies showed a 3–4 fold increase ($p<0.05$) in Ang2 protein overexpression only in the DOX treated TG mice. Albuminuria was increased significantly by 2–3 fold in the TG-DOX treated mice ($p<0.05$) as early as 5 weeks after treatment with DOX. At 10 weeks a significant increase in glomerular extracellular matrix deposition was observed (PAS staining)($p<0.05$).
The expression of vascular endothelial growth factor was reduced in TG DOX treated animals when compared to controls ($p<0.05$).
Conclusion: We have generated a transgenic mouse model that allows podocyte-specific inducible Ang2 overexpression. This resulted in induction of albuminuria and excess extracellular matrix accumulation suggesting a role of Ang2 in renal pathology.
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0164

TGFβ1-mediated activation of protein kinase B/Akt is important for epithelial-mesenchymal transition in diabetic nephropathy

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Background and Aims: The cellular signaling events driving the pathogenesis of diabetic nephropathy are complex and not fully elucidated. Transforming growth factor-beta 1 (TGF-beta1) is a primary mediator of cellular damage during DN, and its upregulation is implicated in epithelial-mesenchymal transition (EMT) associated with DN-associated renal fibrosis. Protein kinase B (PKB/Akt) is a multi-functional regulator of multiple key cellular processes, and some of its downstream targets have been implicated in the onset and progression of EMT in renal fibrosis. Importantly, the regulation of Snail, a transcription factor that represses the expression of e-cadherin during renal fibrosis has been shown to be regulated by GSK3beta, a downstream target of PKB/Akt.
Materials and Methods: In vitro cell culture models of epithelial to mesenchymal transition were used by treated kidney epithelial cells with TGFbeta and/or EGF. CElls were analysed using Western blotting or immunfluoresence.
Results: To investigate the relevance of PKB/Akt activation downstream of TGFbeta1 in tubulointerstitial fibrosis, kidney epithelial cells (NRK-52E and HK2 cells) were exposed to TGFbeta1 for various periods. Phosphorylation of PKB/Akt was evident in cells exposed to TGFbeta1 for 60 min. Activation of downstream targets of PKB/Akt such as GSK3beta and p27Kip1 was also detected. PI3K inhibitors such as LY294002 and wortmannin reduced this TGFbeta1-mediated activation. TGFbeta1 treatment of epithelial cells induces epithelial-mesenchymal transition as judged by decreased levels of epithelial markers e-cadherin and ZO-1, and increased levels of mesenchymal markers such as vimentin and alpha smooth muscle actin. Inhibition of PKB/Akt using LY294002 significantly reduced the ability of TGFbeta1 to induce EMT in these cells, suggesting that PKB/Akt activity is involved in this response.
To examine the role of PKB/Akt activation in DN *in vivo*, we analyzed PKB/Akt activation in kidney sections from both control Wistar and type 2 diabetic Goto–Kakizaki rats (GK). GK rats displayed mild to moderate diabetic

nephropathy at 9–10 months of age. Elevated levels of phosphorylated PKB/Akt and its downstream target pGSK3beta were observed in the kidneys of diabetic, but not control Wistar rats, with pronounced staining in the kidney tubules.

Conclusion: These data suggest that TGFbeta1 mediated PKB/Akt signaling plays an important role in the fibrotic response triggered by the extracellular milieu in diabetic nephropathy.

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0165

Cholecystokinin plays protective roles against progression of diabetic nephropathy through anti-inflammatory effects

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Background and Aims: We have shown that intercellular adhesion molecule-1(ICAM-1) is upregulated and mediates macrophage infiltration into diabetic kidney. ICAM-1 deficiency protects against renal injuries after induction of diabetes suggesting that inflammatory process is involved in the pathogenesis of diabetic nephropathy. DNA microarray analyses using diabetic ICAM-1 knockout (KO) mice suggested that cholecystokinin (CCK) is one of the key molecule for inflammatory process in the progression of diabetic nephropathy. CCK is a peptide with various functions including regulation of inflammatory reaction, gastrointestinal homeostasis, and neurotransmittion. Two types of CCK receptors, types A and B, have been identified. Both type receptors are expressed in macrophage and kidney. The aim of this study is to clarify the role of CCK in the pathogenesis of diabetic nephropathy using 5/6 nephrectomy which is the model of non-immune glomerular sclerosis.

Materials and Methods: 1) We performed DNA microarray analysis using the kidneys from diabetic and non-diabetic ICAM-1 KO (ID and IND) and wild type mice (WD and WND). We also used 5/6 nephrectomized and sham-operated ICAM-1 KO (IN and IS) and wild type mice (WN and WS). **2)** We performed 5/6 nephrectomy or sham operation on CCK-A receptor KO (AN), CCK-B receptor KO (BN), CCK-A and B receptor double KO (ABN) mice and wild type mice. We evaluated renal function, blood pressure and renal pathology over a period 3 month. Glomerular size and mesangial matrix area were measured by morphometry using NIH Image. DNA microarray analysis (GeneChip™,Affimetrix) was performed with RNA from the kidney of ABN and WN. **3)** Cultured macrophages (PMA-treated THP-1 cells) were treated with sulfated CCK octapeptide (CCK-8s), control peptide and vehicle for 24 h. Expression of TNF-α, IFN-γ , IL-1β and MCP-1 in the 3 groups of macrophages was measured by quantitative real time RT-PCR.

Results: 1) Signal intensity of CCK was increased in WD or IN compared with WND or WS, and decreased in ID or IN compared with WD or WN. CCK was detected in renal tubules by immunoperoxidase staining. **2)** Blood pressure was not different in the 8 groups. Creatinine clearance (Ccr) was lower in ABN than in WN. The glomerular size was significantly larger in ABN than WN ($p<0.05$). Mesangial matrix area was significantly greater in ABN than in the WN ($p<0.05$). AN and BN had no significant difference from WN in Ccr and morphometry. DNA microarray indicated that 93 genes were upregulated and 101 genes were downregulated in ABN as compared with WN. These genes were classified into functional Gene Ontology categories. The analysis indicated that the category of the most significantly upregulated genes was “immune response” including inflammatory related genes ($p<0.001$). **3)** Cultured macrophages treated with CCK-8s suppressed mRNA expression of TNF-α as compared with control peptide and vehicle.

Conclusions: CCK mRNA expression was increased in diabetic and 5/6 nephrectomized kidney. Deficiency of both CCK-A and B receptors accelerated renal injuries as compared with wild type mice. Inflammatory related gene expression was upregulated in the kidney of ABN as compared with WN. CCK-8s inhibited the production of inflammatory cytokines from cultured macrophages. CCK may play protective roles against progression of diabetic nephropathy through anti-inflammatory effects.

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0166

Proinsulin C-peptide constricts afferent glomerular arterioles in diabetic mice. A potential reno-protective mechanism of action

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Background and Aims: The initial phase of diabetes is commonly associated with glomerular hyperfiltration filtration, and this has been postulated to be involved in the progression of diabetic nephropathy. Several studies have reported reno-protective effects of C-peptide in both diabetic subjects and in animal models. Furthermore, C-peptide has been shown to decrease the diabetes-induced hyperfiltration in insulin-dependent diabetic subjects. In this study, we investigated the vascular reactivity to C-peptide in isolated afferent arterioles from control and alloxan-induced diabetic C57/Bl mice.
Materials and Methods: Diabetes was induced by an intravenous injection of alloxan (75 mg/kg bw) two weeks prior to the experiment. Age-matched normoglycemic mice served as control. Afferent arterioles, with intact glomerulus, were isolated and fixed in a confocal microscope. The arterioles were perfused with DMEM, and thereafter with DMEM containing C-peptide (1 nM). All results were compared to that of vehicle and a scrambled peptide of identical size as C-peptide, but with no known physiological function.
Results: The blood glucose level was elevated in the alloxan-treated mice (18.2±0.6 vs. control 6.4±0.2 mmol/l). C-peptide reduced the diameter of the afferent arterioles from diabetic mice (final reduction −27±8%; n=7) within 10 minutes, whereas arterioles from control animals first showed a modest response to C-peptide after 30 minutes (−4±1; n=7). The vehicle or the scrambled peptide did not have any effect in either group.
Conclusion: Our findings show that C-peptide has a pronounced constricting effect on afferent arterioles isolated from diabetic mice, whereas it has only a modest and delayed effect on arterioles originating from normoglycemic control mice. This suggests that the mechanism, by which C-peptide reduces the diabetes-induced glomerular hyperfiltration, is to constrict the afferent arteriole, thereby reducing the glomerular filtration pressure. Thus, new treatment strategies taking into account the beneficial effects of C-peptide could potentially reduce the prevalence and incidence of diabetic nephropathy.

0167

The role of the angiotensin II type 2 receptor subtype in the development and progression of end-organ injury in experimental diabetes

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Background: Although many of the renal and cardiac actions of angiotensin II (Ang II) are mediated via the type 1 angiotensin (AT1) receptor subtype, there is evidence that under pathophysiological conditions, the type 2 (AT2) receptor subtype may also be an important regulator of the response to injury. However, the status and role of the AT2 receptor in the diabetic context has not been fully characterised.
Aims: The aims of this study were firstly to assess the renal and cardiac expression of the AT2 receptor subtype (with concomitant measurement of AT1 receptor expression) in long term diabetes and secondly to evaluate the effect of an AT1 receptor antagonist on AT2 receptor expression.
Methods: Diabetes was induced by injection of streptozotocin (55 mg.kg-1) in 6-week-old C57Bl6 and Apo E deficient (a model of accelerated renal and vascular complications) mice and further randomised to no treatment or to the AT1 receptor antagonist, irbesartan (10 mg.kg-1) for 20 weeks (n=6–8/group). The status of the AT2 receptor was assessed by real time RT-PCR, immunohistochemistry and *in vitro* autoradiography.
Results: The induction of diabetes was associated with increased expression of the AT2 receptor at sites of end-organ injury, including the kidney (control, 1.0±0.2 vs diabetic C57, 6.2±1.8, $p<0.05$) and the heart (control, 1.0±0.4 vs diabetic C57, 18.6±4.9, $p<0.01$). A similar pattern was seen in diabetic apo E deficient mice with an approximately 7 and 25 fold increase in AT2 receptor expression in the kidney and heart respectively. Treatment with the AT1 receptor antagonist, which attenuates end-organ injury at all sites in these 2 models, ameliorated the diabetes associated increase in AT2 receptor expression (renal, diabetic C57+irbesartan, 1.2±0.8, $p<0.05$ vs untreated diabetes). A similar reduction in AT2 receptor expression was also seen in both the kidney and heart in diabetic apoE deficient mice treated with irbesartan. In the same organs, the gene expression of the AT1 receptor was reduced or unchanged by diabetes and was in general upregulated>100% by irbesartan treatment.
Conclusions: There was increased cardiac and renal expression of the AT2 receptor subtype in experimental diabetes. Whether this represents a protective and/or pathogenic response in diabetes remains to be established by further studies in either AT2 deficient mice or mice treated with an AT2 receptor antagonist.
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0168

Tight control of arterial pressure and not inhibition of renin-angiotensin system determines prevention of glomerular hypertrophy and nephrin loss in experimental diabetes

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Background and Aims: A recent systematic review and meta-analysis on the effect of inhibitors of the renin–angiotensin system in diabetic renal disease have concluded that: "additional renoprotective actions of these substances beyond lowering blood pressure remain unproven" (Casas et al. Lancet 2005;336:2026). The present study tested the hypothesis that in spontaneously hypertensive rats (SHR) with streptozotocin-induced diabetes mellitus (DM) strict control of arterial pressure with or without inhibition of the renin–angiotensin system can prevent the development of glomerular hypertrophy and the reduction in renal expression of nephrin.
Materials and Methods: DM was induced by injecting streptozotocin in 4-week-old, prehypertensive, SHR and the rats were randomized for no treatment, or for treatment with captopril, losartan or triple therapy (hydrochlorothiazide, reserpine and hydralazine) for 20 days.
Results: Plasma glucose levels in treated and non-treated diabetic rats were consistently and significantly elevated (>20 mmol/l, $p<0.0001$) when compared to control SHR (about 8 mmol/l). The increase in systolic blood pressure was equally prevented by captopril (115±16 mmHg), losartan (108±12), and triple therapy (113±9). Albuminuria was higher in diabetic rats [400 (111–501) μg/24 h] and similarly reduced ($p<0.05$) by captopril [210 (68–449)], losartan [190 (70–543)], and triple therapy [171 (62–381)]. Glomerular expression of nephrin, estimated by Western blot and immunofluorescence, was reduced in diabetic SHR in comparison with controls and the antihypertensive treatment prevented the reduction in glomerular expression of nephrin. Glomerular size was higher ($p<0.005$) in diabetic SHR (27,300±2130 μm2) compared with non-diabetic SHR (23,800±307). The antihypertensive therapy with captopril (23,900±175), losartan (23,800±120), and triple therapy (23,400±210) prevented the glomerular enlargement in diabetic SHR. Glomerular expression of fibronectin and TGF-β IIR were increased in diabetic SHR and they were normalized by treatment with the antihypertensive drugs.
Conclusion: In diabetic SHR rats strict control of arterial pressure with or without inhibition of the renin–angiotensin system can prevent the development of glomerular hypertrophy and reduction in renal expression of nephrin.
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OP 29

Incretin hormone action – animal studies

0169

The classic incretin receptors for GLP-1 and GIP are essential for the sustained glucoregulatory actions of vildagliptin in mice

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Background and Aims: DPP-4 inhibitors (DPP-4i) lower blood glucose and HbA1c in experimental models and in clinical therapy of human subjects with type 2 diabetes, however the mechanism of action through which these agents improve glucose homeostasis remains incompletely understood. Although GLP-1 and GIP represent the predominant substrates mediating glucose reduction following acute DPP-4 inhibition, whether sustained inhibition of DPP-4 activity exerts anti-diabetic actions via recruitment of additional glucoregulatory substrates such as GRP or PACAP remains uncertain. To determine whether additional substrates contribute to the antidiabetic actions of DPP-4i, we examined the efficacy of LAF-237 (Vildagliptin) in high fat (HF)-fed wildtype (WT) or dual incretin receptor knockout (DIRKO) mice over 8 weeks.
Results: Vildagliptin had no significant effect on food intake, energy expenditure, body composition, body weight gain or insulin sensitivity, as assessed by ITT, in WT or DIRKO mice. Although Vildagliptin had no effect on fasting glucose, glycemic excursion following OGTT was significantly reduced in WT but not in DIRKO mice after 7 weeks of drug treatment. Pancreatic insulin content was comparable in mice with or without 8 weeks of Vildagliptin therapy. Vildagliptin increased levels of glucose-stimulated plasma insulin, acutely decreased levels of plasma glucagon, and reduced levels of cholesterol in WT but not in DIRKO mice. Furthermore, Vildagliptin therapy was associated with reduced hepatic expression of genes important for cholesterol synthesis. As GIP exerts complex actions on fat tissues, we analyzed adipokine expression in different fat depots. Although Vildagliptin had no effect on levels of epididymal or inguinal fat pad adipokine mRNA transcripts for adiponectin, leptin, resistin, TNF-alpha, IL-

1beta, and IL-6 WT mice, PAI-1 mRNA transcripts were increased in WT yet significantly decreased in epididymal fat pads from DIRKO mice following 8 weeks of Vildagliptin therapy

Conclusion: These findings illustrate that 8 weeks of continuous DPP-4 inhibition improves glucose homeostasis and parameters of cholesterol synthesis in WT but not in DIRKO mice. Hence classic incretin receptors represent the dominant molecular mechanisms for transducing the chronic anti-diabetic actions mediated by DPP-4 inhibition *in vivo*.

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0170

CJC-1134, an exendin-4-albumin GLP-1R agonist potently activates GLP-1R-dependent actions controlling glucose homeostasis

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Background and Aims: GLP-1 lowers blood glucose via multiple actions however the therapeutic potential of native GLP-1 is limited by its short plasma half-life, due to rapid inactivation by DPP-4 and renal clearance. We have now examined the biological actions of CJC-1134, a conjugate of exendin-4 covalently linked, ex vivo, to human serum albumin, to determine if much larger peptide-albumin hybrid molecules can exert similar actions to those exhibited by native exendin-4.

Materials and Methods: The acute actions of CJC-1134 were examined in both wild-type C57BL/6 and GLP-1 receptor−/− (GLP-1R−/−) mice. In longer–term studies, C57BL/6 mice were maintained on a high fat diet (HFD; 45% kcal fat) for an initial 4 weeks followed by an additional 4 week period of high fat feeding in the presence of ip injections of (i) twice daily saline, (ii) twice daily exendin-4 (Ex-4; 24 nmol/kg), (iii) once daily human serum albumin (HSA; 100 nmol/kg), or (iv) once daily CJC-1134 (100 nmol/kg).

Results: CJC-1134 dose-dependently lowered the glycemic excursion in response to either oral or ip glucose challenge following acute administration in mice. Although larger GLP-1R agonists may not penetrate the CNS to the same extent as smaller peptides like GLP-1 or exendin-4, both CJC-1134 and exendin-4 produced comparable inhibition of gastric emptying (PBS: 70.7±3.9% vs. Ex-4: 11.4±6.6%, $P<0.001$; HSA: 75.7±5.8% vs. CJC-1134: 23.3±4.0, $P<0.001$). CJC-1134 also reduced 24-hr food intake, and activated c-fos expression in the murine CNS in wild-type mice. Furthermore, consistent with the GLP-1R-dependent specificity of CJC-1134 action, CJC-1134 did not lower blood glucose, inhibit gastric emptying or activate CNS c-fos expression in GLP-1R−/− mice. Chronic administration of exendin-4 or CJC-1134 significantly reduced body weight (PBS: 33.9±0.9 g vs. Ex-4: 28.8±0.5 g, $P<0.001$; HSA: 33.7±0.7 g vs. CJC-1134: 27.8±0.5 g, $P<0.001$), epididymal fat pad weight (PBS: 1.68±0.16 g vs. Ex-4: 0.68±0.07 g, $P<0.001$; HSA: 1.42±0.18 g vs. CJC-1134: 0.61±0.06 g, $P<0.001$), and total fat mass (PBS: 10.5±0.7 g vs. Ex-4: 4.7±0.7 g, $P<0.05$; HSA: 6.9±1.1 g vs. CJC-1134: 2.4±0.4 g, $P<0.05$) and increased total pancreas weight (PBS: 0.28±0.01 g vs. Ex-4: 0.33±0.01 g, $P<0.01$; HSA: 0.30±0.01 g vs. CJC-1134: 0.38±0.01 g, $P<0.001$). CJC-1134 also significantly decreased HbA1c levels (HSA: 3.99±0.12% vs. CJC-1134: 3.58±0.05%, $P<0.01$). Moreover, both exendin-4 and CJC-1134 reduced the glucose excursion in response to ip or oral glucose challenge performed after 3 or 4 weeks of drug treatment, respectively. Plasma cholesterol was reduced in CJC-1134-treated mice (HSA: 145.9±8.6 mg/dl vs. CJC-1134: 120.9±5.5 mg/dl, $P<0.05$), and hepatic triglyceride content was significantly lower in mice treated with either exendin-4 or CJC-1134 (PBS: 7.6±1.2 mg/g vs. Ex-4: 5.5±0.5 mg/g, $P<0.05$; HSA: 6.0±0.7 mg/g vs. CJC-1134: 3.4±0.4 mg/g, $P<0.01$). The effects of once-daily CJC-1134 were greater than those observed for twice-daily exendin-4 for the majority of GLP-1R-dependent endpoints examined

Conclusion: These studies illustrate that the larger exendin-4:albumin conjugate CJC1134 can elicit the same profile of GLP-1R-dependent biological actions, both in the periphery and CNS, relative to that obtained with smaller GLP-1R agonists such as exendin-4. The extended pharmacokinetic profile of albumin-based therapeutics in human subjects raises the possibility that CJC-1134 may be suitable for once weekly dosing for the treatment of type 2 diabetes.

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0171

Early administration of the GIP receptor antagonist (Pro^3)GIP counters development of hyperglycaemia, glucose intolerance and insulin resistance in genetic obesity

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Background and Aims: Gastric inhibitory polypeptide (GIP) is a gastrointestinal hormone secreted in response to feeding that stimulates insulin secretion and regulates carbohydrate and lipid metabolism. The actions of GIP are blocked by (Pro^3)GIP, a well-characterised stable and specific GIP receptor antagonist. This study utilised (Pro^3)GIP to inves-

tigate the effects of longer-term (60-day) GIP receptor ablation on development of spontaneous obesity and diabetes in young obese (*ob/ob*) mice.

Materials and Methods: (Pro^3)GIP was synthesised using solid-phase Fmoc peptide chemistry and structure was confirmed by matrix assisted laser desorption ionisation mass spectrometry. Obese (*ob/ob*) mice (5–7 weeks) were divided into two groups (n=8) that received, once daily i.p. injections (17:00 h) over a 60-day period, of either saline vehicle (0.9%, w/v, NaCl) or (Pro^3)GIP (25 nmol/kg body weight). Food intake and body weight were recorded five days before and daily throughout the study period. Plasma glucose and insulin concentrations were measured at 4–7 day intervals. At the end of the treatment period (day 60), glucose tolerance (18 mmol/kg glucose i.p.), insulin sensitivity (50 U insulin/kg bw), glycated haemoglobin (%HbA_{1c}) and plasma lipidse were assessed.

Results: Weight gain was suppressed in (Pro^3)GIP treated mice, but body weights and food intake did not significantly differ between groups at 60-days. However, non-fasting plasma glucose levels were significantly decreased in mice receiving (Pro^3)GIP treatment from day 13 (10.8±0.5 mmol/l *vs*. 12.9±0.7 mmol/l for control; P<0.05), did not rise thereafter and remained significantly lower throughout the entire study (P<0.05 to P<0.001). Circulating plasma insulin levels were also significantly reduced in (Pro^3)GIP treated mice, being 45.9±4.5 ng/ml *vs*. 73.1±8.9 ng/ml for control (P<0.05) on day 44. Following i.p. glucose administration on day 60, the overall plasma glucose excursion was significantly (1.9-fold; P<0.001) lowered in (Pro^3)GIP treated mice (712.8±73.8 mmol/l.min *vs*. 1339.0±64.4 mmol/l.min for control). Plasma insulin concentrations were also significantly diminished 15 min post glucose injection (37%; P<0.05), which was reflected by a significantly 2.1-fold reduced (P<0.05) overall insulinotropic response in (Pro^3)GIP treated mice. Exogenous insulin injection caused a significantly greater fall of glucose concentrations in (Pro^3)GIP treated *ob/ob* mice (105.9±17.5 mmol/l.min *vs*. 145.5±32.0 mmol/l.min for control; P<0.05) indicative of substantial alleviation of insulin resistance. In keeping with improved glycaemic control, (Pro^3)GIP treated mice had significantly (P<0.05) decreased %HbA_{1c} (4.5±0.4%) levels compared to controls (6.0±0.3%). Assessment of plasma lipids revealed that 60-day (Pro^3)GIP treated mice had significantly lower triglyceride (122.3±9.6 mg/dl *vs*. 150.1±4.9 mg/dl for control; P<0.05) and LDL-cholesterol concentrations (1.5-fold; P<0.05) compared to controls.

Conclusion: Once daily administration of (Pro^3)GIP for 60 days improved metabolic regulation and countered development of diabetes in *ob/ob* mice. This illustrates the potential of (Pro^3)GIP for treatment of obesity-related type 2 diabetes, acting through beta cell sparing and the alleviation of insulin resistance.

0172

Long-acting analogues of GIP with significantly improved antihyperglycaemic and insulinotropic properties: N-terminal modification versus mid-chain acylation

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Background and Aims: Glucose dependent insulinotropic polypeptide (GIP) is an insulinotropic gastrointestinal hormone with therapeutic potential for type 2 diabetes. However, metabolic instability and rapid renal clearance limit the development of GIP-based therapies. Numerous GIP analogues have now been generated exhibiting DPP IV resistance and extended bioactive profiles. The present study reports a direct evaluation of the long-term antidiabetic actions of three such GIP molecules, *N*-AcGIP, GIP(Lys^{37}PAL) and *N*-AcGIP(Lys^{37}PAL) in obese diabetic (*ob/ob*) mice.

Materials and Methods: GIP-related peptides were synthesised using solid-phase Fmoc peptide chemistry and structure confirmed by matrix assisted laser desorption ionisation mass spectrometry. Obese–diabetic (*ob/ob*) mice (12–15 weeks) were divided into four groups (n=8) that received, once daily i.p. injections (17:00 h) over a 14-day period, of either saline vehicle (0.9%, w/v, NaCl), *N*-AcGIP, GIP(Lys^{37}PAL) or *N*-AcGIP(Lys^{37}PAL) (all at 25 nmol/kg body weight). Food intake and body weight were recorded five days before and daily throughout the 14-day study period. Plasma glucose and insulin concentrations were measured at 3–4 day intervals. At the end of the treatment period, circulating glucagon, glucose tolerance (18 mmol/kg glucose), insulin sensitivity (50 U insulin/kg bodyweight), response to GIP (25 nmol/kg body weight), pancreatic insulin content and islet morphology were assessed.

Results: Body weight, food intake, circulating glucagon and insulin did not significantly differ (P>0.05) between groups during the study. However, on day 14 of treatment, non-fasting plasma glucose levels were significantly (P<0.05) reduced in all GIP analogue treated groups compared to control (12.0–12.8±1.2 mmol/l *vs*. 15.0±0.6 mmol/l.min for control). Following i.p. glucose administration, the overall plasma glucose excursion was significantly lowered in the 14-day *N*-AcGIP (1.3-fold; P<0.01), GIP(Lys^{37}PAL) (1.2-fold; P<0.05) and *N*-AcGIP(Lys^{37}PAL) (1.2-fold; P<0.05) treated mice compared to saline control. In harmony with this, all three GIP analogues significantly enhanced glucose-induced insulin release (P<0.05). Exogenous insulin injection caused a significantly greater fall of glucose concentrations in *N*-AcGIP, GIP(Lys^{37}PAL) and *N*-AcGIP(Lys^{37}PAL) treated *ob/ob* mice

compared to control (118.0–126.6±13.8 mmol/l.min *vs*. 174±14.4 mmol/l.min for control; P<0.05 in all cases). The metabolic and insulin secretory responses to native GIP were also significantly (P<0.05–P<0.01) enhanced in all 14-day analogue treated mice compared to control, revealing no evidence of GIP-receptor desensitization. These effects were accompanied by a significant increase in pancreatic islet area (0.029–0.031±0.0038 mm^2 *vs*. 0.021±0.0027 mm^2 for control; P<0.01 in all cases) and islet number (1.4–1.6 fold; P<0.05 – P<0.01) in all GIP analogue groups.

Conclusion: These data demonstrate the therapeutic potential of once daily injection of enzyme resistant GIP analogues and suggest that *N*-AcGIP is equally as effective as related palmitate derivatised analogues of GIP, GIP (Lys^{37}PAL) and *N*-AcGIP(Lys^{37}PAL).

0173

Liraglutide, a long-acting GLP-1 analogue, expands the β-cell mass of human pancreatic cells implanted in immunodeficient diabetic mice and corrects their diabetes: gastrin potentiates liraglutide's effects

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Background and Aims: Pancreatic islet transplantation is a viable treatment for type 1 diabetes, but is limited by human donor tissue availability. In this study we investigated whether the β-cell mass in human pancreatic cell preparations with low endocrine cell purity could be expanded by treating the cells with liraglutide, a long-acting glucagon-like peptide-1 (GLP-1) analog and gastrin, peptides reported to induce β-cell neogenesis in diabetic animal models.

Materials and Methods: Islets were isolated from pancreases of adult human brain-dead organ donors, dissociated and 5×10^{6} cells (7±2% insulin+β cells, 37±7% CK 7^{+}+CK19+duct cells, and 11±2% amylase+acinar cells) were implanted under the left renal capsule of nonobese diabetic-severe combined immune deficiency (NOD–scid) mice made diabetic (blood glucose 20–25 mmol/l) with streptozotocin. The mice were injected daily with vehicle, a low dose of liraglutide 100 ug/kg/day, gastrin 300 ug/kg/day, and liraglutide+gastrin. In the first study, blood glucose (BG) was measured weekly, and after 5 weeks the human pancreatic cell grafts were harvested and dissociated and their cell compositions were analyzed by immunocytochemistry. In the second study, BG was measured weekly, and after 4 weeks plasma human C-peptide was measured by RIA ; then the left kidneys with the human pancreatic cell grafts were removed and insulin contents were measured by ELISA, and the mice were monitored daily for return of hyperglycemia.

Results: After 5 weeks, BG rose to 27.1±1.6 mmol/l in vehicle – treated mice (n=9), and decreased to 18.7±1.8 mmol/l in gastrin–treated mice (n=12); 11.8±1.9 mmol/l in liraglutide–treated mice (n=10) and 7.9±0.9 mmol/l in liraglutide+gastrin treated mice. BG was not decreased in sham–implanted mice receiving any treatment. β-cells in the human pancreatic cell implants increased from 2.38±0.45×10^{5} cells to 3.99±0.79×10^{5} cells in vehicle–treated mice, 7.72±1.23×10^{5} cells in gastrin–treated mice, 11.65±0.96×10^{5} cells in liraglu tide–treated mice, and 16.10±2.12×10^{5} cells in liraglutide+gastrin–treated mice. Insulin content in the human pancreatic cell implants in the mice decreased from 126±17 ng to 14±1 ng after 5 weeks in vehicle–treated mice, but increased to 288±52 ng in gastrin–treated mice, 459±123 ng in liraglutide–treated mice and 1105±257 in liraglutide+gastrin–treated mice. Correction of hyperglycemia by the peptides correlated with the insulin content in the human pancreatic cell implants (r=−0.978) and with the plasma human C-peptide level (r=−0.862) in the mice. Diabetes (BG≥20 mmol/l) recurred within a few days after removal of the implants.

Conclusion: These results show that a low dose of liraglutide expands the β-cell mass of adult human pancreatic cell preparations of low endocrine cell purity after implantation in immunodeficient diabetic mice. The increase in β-cells is 5-fold and sufficient to correct hyperglycemia in the diabetic mice. Gastrin potentiates these effects of liraglutide.

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0174

Differential effects of DPP-4 inhibition on incretin hormone levels in drug-naive and metformin-treated patients with type 2 diabetes

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Background and Aims: Vildagliptin is an inhibitor of DPP-4, the enzyme that degrades the incretin hormones GLP-1 and GIP. Vildagliptin lowers fasting (FPG) and postprandial (PP) glucose in patients (pts) with type 2 diabetes (T2DM) via incretin hormone-mediated improvements in islet function. Because metformin (Met) has been suggested to increase GLP-1 release, we sought to determine if the effect of

vildagliptin on plasma levels of intact (active) GLP-1 and GIP was greater in patients (pts) with T2DM receiving concomitant Met than in pts receiving vildagliptin alone.
Methods: Three randomized, double-blind, placebo (PBO)-controlled studies were performed and plasma levels of active GLP-1 and GIP were measured by ELISA and RIA, respectively, using N-terminally directed antisera. In Study 1, GLP-1 and GIP were measured in overnight-fasted pts before (Wk 0) and after 12-wk treatment with vildagliptin 50 mg bid. In Study 2, PP GLP-1 and GIP were measured after a single 100 mg dose of vildagliptin or PBO given before a meal. In Study 3, fasting and PP GLP-1 and GIP were measured after 6-wk treatment with vildagliptin 50 mg bid or PBO. Each study included pts continuing a stable Met regimen (Met) and pts receiving vildagliptin alone (No Met).
Results: As shown below, in Study 1, vildagliptin significantly increased fasting levels of active GLP-1 in the Met group, but did not affect fasting GIP. In the No Met group, fasting GIP increased, but fasting GLP-1 did not. In Study 2, vildagliptin increased the 2-h mean PP levels (AUC ÷ time) of active GLP-1 and GIP in both subgroups, but PP GIP was higher in the No Met vs the Met subgroup. In Study 3 vildagliptin significantly increased fasting levels of active GLP-1 only in the Met subgroup, but it did not significantly affect fasting GIP in either subgroup. In Study 3, vildagliptin increased PP levels of the active forms of both GLP-1 and GIP, but the increase in PP GLP-1 was significantly greater in the Met than in the No Met subgroup.

	Active GLP-1 (pM)		Active GIP (pM)	
	No Met (n=7)	Met (n=12)	No Met (n=7)	Met (n=12)
Study 1				
Wk 0, Fasting	5.7±2.2	2.3±0.1	16±2	17±2
Wk 12, Fasting	7.3±3.4	12.5±4.9[a]	25±5[a]	19±3
Study 2				
	No Met (n=4)	Met (n=8)	No Met (n=4)	Met (n=8)
PBO, 2-h mean PP	3.3±0.8	5.2±0.8	42.4±7.8	36.1±4.2
Vildagliptin, 2-h mean PP	7.4±0.9[b]	10.8±1.4[b]	79.0±7.0[b]	56.1±3.0[b,c]
Study 3				
	No Met (n=5)	Met (n=12)	No Met (n=5)	Met (n=12)
PBO, Fasting	2.8±0.8	3.4±1.0	2.4±0.2	11.3±1.5
Vildagliptin, Fasting	4.1±1.4	11.3±2.9[b]	7.8±3.0	13.5±3.0
PBO, 2-h mean PP	5.0±1.5	8.6±2.4	14.2±3.5	23.5±3.1
Vildagliptin, 2-h mean PP	9.7±1.7[b]	22.7±3.1[b,c]	34.7±6.9[b]	40.0±3.6[b]

[a]P<0.05 vs Wk 0; [b]P<0.05 vs PBO; [c]P<0.05 vs No Met
Values below limit of quantification (LOQ) set equal to LOQ

Conclusions: DPP-4 inhibition with vildagliptin has a larger effect on active GLP-1 in pts receiving concomitant Met than in pts receiving no concomitant antidiabetic agent. In pts receiving no Met there is a more pronounced effect on active GIP. Although both GLP-1 and GIP enhance insulin release, GIP stimulates, but GLP-1 suppresses glucagon release. Thus DPP-4 inhibition may also have differential effects on α- and β-cell function in drug-naïve vs Met-treated pts. The more robust effect on GLP-1 (vs GIP) in Met-treated pts may underlie the particularly pronounced efficacy of vildagliptin to reduce both FPG and HbA_{1c} in Met-treated pts.
Supported by Novartis

OP 30

Diabetes in childhood

0175

Alarming continued increase of overweight at onset of insulin-treated diabetes in both African–American and Caucasian children

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Background and Aims: Insulin-treated diabetes in childhood was thought to be a disease of wasting, not overweight. We have previously reported that the prevalence of being overweight more than tripled from 1979–1989 (period I) to 1990–1998 (period II) at onset of islet autoantibody positive childhood insulin-treated diabetes — the concept of "double" diabetes. The current study evaluates whether this increase has continued in the period 1999–2002 (period III).
Materials and Methods: All Black children <19 years of age, diagnosed with diabetes and treated with insulin at onset, admitted to Children's Hospital of Pittsburgh from January 1st 1979 to December 31st 2002 were matched with White children by sex, age at onset and year of diagnosis. Data were obtained from retrospective review of medical records and β-cell antibodies measured at onset. Overweight was defined as body mass index≥85th percentile for age and sex.
Results: Complete data were available for 252 children out of 332 (76%). Overall, the prevalence of being overweight in all 252 insulin treated subjects more than tripled in 24 years of observation, 12.6% in period I to 36.8% in period II, to 43.9% in period III (p=0.0005). In period II,

the effect was greatest in Black females <11 years (0% to 50% to 25% in periods I II and III respectively, p=0.04). In period III, the biggest increase was seen in those previously less affected, i.e. younger White males (0% to 9.1% to 37.5% in periods I, II and III respectively, p=0.05). The prevalence by race, sex and age at onset is seen in the table below.

	<11 years (n=108)			>=11 years (n=144)		
	Period I	Period II	Period III	Period I	Period II	Period III
Whites (n=124)	5.0	4.8	25*	0	25.8	33.3**
Blacks (n=128)	5.9	31.8	41.8*	38.9	68.4	67.7*
Males (n=117)	5.2	9.5	44.4***	29.4	59.2	50
Females (n=135)	5.5	25	12.5	11.7	42.8	50**

*p=0.08; **p<0.05; ***p<0.005 comparing the three periods by age

There was no significant difference in terms of frequency of ketoacidosis between the three periods (36.5% vs 37% vs 31.2% respectively, p=0.71). The frequency of addition of metformin to the treatment regimen with insulin increased from period I (2.8%) to period II and III (14.4% and 11.9% respectively, p=0.04).

Conclusion: The phenotype of children with insulin-treated diabetes at onset is changing. Almost half of the children with insulin-treated diabetes are now overweight despite frequent onset weight loss. The recent rise is greatest in younger white males. It is yet to be determined whether this weight excess is related to the continued increase in type 1 diabetes incidence or if it affects rates of complications. Lifestyle intervention studies in high risk populations (e.g. first degree relatives) are needed to assess potential beneficial effects in prevention/delay of the onset of the disease or its comorbidities.

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0176

An attempt of pancreatic islet beta-cells prevention after clinical diagnosis of type 1 diabetes mellitus

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Backgroud: In recent years studies focused on prevention of type 1 diabetes mellitus (DM) and possibility of beta-cell protection at the clinical diagnosis of DM. Some recent studies have demonstrated beneficial role of vit. D3 analogue in human beta-cell activity maintenance at the early stages of DM.

Material and Methods: The study was performed in 63 children and adolescents (32 female and 31 male) aged 4–16 years who received insulin and vit D3 analogue (alfacalcidol – 1alpha–OH–vitamin D3) in daily dose 0.25 μg. 37 peers (17 female and 20 male) with type 1 DM who received only insulin therapy without vit. D3 administration were the control group. After one year therapy we compared the level of HbA1c, body mass, daily insulin dose in both groups.

Results: There are no differencies in mean body mass and in metabolic control of diabetes reported by mean HbA1c level at the onset of the DB and after one year observation of these patients among studied groups. However, daily insulin dose in group of patients who received vit D3 therapy after one year observation were at the similar level compared to the early stage of DM (0.36 u/kg – 0.37 u/kg). On the contrary, insuline requirement in the group of patients without vit D3 treatment significantly increased after one year observation (from 0.37 u/kg to 0.63 u/kg).

Conclusion: In group of children treated alfacalcidol insulin requirement after one year therapy with vit D3 analogue sustained at the level found at the early stage of DM. Children without vit D3 administration had twofold incerase of insulin requirement after one year observation from clinical diagnosis. Our observations suggest, that alfacalcidol could play a role in prevention of further beta-cells destruction.

0177

Post-prandial glucose excursions following three methods of bolus insulin administration in adolescent girls with type 1 diabetes

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Objectives: To determine if one method of short-acting insulin bolus administration is superior to other methods in managing a meal with the same content of calories and carbohydrates but with different content of fat.

Methods: Thirteen girls aged 13–19 years with type 1 diabetes agreed to consume two different lunch meals on 6 occasions with one week apart. The meals were exactly identical with the same content of calories and carbohydrates but with 30 or 36% fat. They received the same dose of bolus insulin on each of the 6 occasions as a single dose immediately prior to the meal as a single bolus (N), a dual-

wave (60% as a single bolus and 40% as square wave over 1 h,DF) or as a square wave over 1 h(F). The plasma–glucose levels were followed with CGMS every fifth minute between 30 min before and until 3 h after the meal.
Results: The maximal change of plasma–glucose occurred after 110 min (2,7 mmol), 85 min (2,6 mmol) and 55 (3,8 mmol) min after the 30% fat meal with the N, DF and F bolus administration. The maximal change of plasma–glucose after the 36% fat meal with the N, DF and F bolus after 75 min(2,6 mmol), 45 min(1,8 mmol) and 65 min (2,8 mmol) The area under the curve between start and 180 min was lowest after the DF bolus for both types of meals ($p<0,001$).
Conclusions: The dual wave method for bolus administration seams to be the most effective method for a recommended type of main-meal or a "pizza"-type of meal.
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0178

Contribution of basal insulin on daily dose is related to c-peptide secretions in diabetes children treated with continuous subcutaneous insulin infusion

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Background and Aims: Continuous subcutaneous insulin infusion has become an increasingly used option in type 1 diabetes children management. This advanced technology provides the most precise insulin administration and also allows to keep the records of delivered insulin doses over the period of last 12 weeks. The analysis of registered data gives an opportunity to review obligatory algorithms of insulin adjustment in intensive therapy.
The aim was to investigate the insulin requirement, basal insulin contribution on total insulin daily dose (TIDD) and analysis key determinant factors for basal to bolus proportion in TIDD.
Materials and Methods: 78 (35 female/43 male) well controlled type 1 diabetes children were included in the study; mean age 10,19±4,19 y (1,05–17,91), mean diabetes duration 3,38±SD 2,7 y (0–12,7), HbA1c 6,75±0,84%, BMI 17,93±SD2,78 kg/m2. During hospital visits pumps memory data was downloaded; clinical and biochemical: fasting c-peptide, GlyHbA1c were assessed. Patients with DKA (ketoacidosis), SH(severe hypoglycemia) or infection diseases during last 3 months were excluded.
Results: The average requirement for insulin was 0,75±0,21 U/kg/d, and it was not significantly different in age groups: it was comparable in prepubertal and pubertal children (0,71±0,17 vs 0,79±0,24 U/kg/d) but basal contribution was statistically lower in prepubertal patients than in pubertal (22,35±8,76 vs 31,23±14,82% $p<0,05$). Analysis of factors of basal contribution on TIDD shows significant correlation with age ($r=0,33$ $p=0,05$), diabetes durations ($r=0,54$, $p<0,0001$) and BMI ($r=0,325$ $p<0,0037$) but inversely correlated with C-peptide ($r=-0,349$ $p=0,0017$). However C-peptide did not significantly correlated with TIDD.
Conclusion: The basal to bolus insulin is below 50% of TIDD in children with type 1 diabetes. The basal contribution in growing-up population should be differentiated by the age, diabetes duration and BMI. Remission phase related to residual secretion of insulin by beta cell, clinically is expressed by contribution of basal insulin on TIDD but not by insulin amounts per kg of body mass per day.

0179

Risk for hospitalisation in diabetic children and adolescents and its association with social status

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Background and Aims: Hospitalization in type 1 diabetic children and adolescents is high, despite of many efforts to reduce inpatient care in chronic diseases. Children from socially deprived families seem to have a disproportionately high risk to be hospitalized. However, data regarding the association between hospitalization risk and social status are limited, and results are conflicting. Thus, aim of our study was to evaluate the association between post-onset hospitalization and the social situation in a large cohort of newly diagnosed type 1 diabetic children and adolescents, based on data of a nationwide established documentation program (DPV).
Materials and Methods: From 62 pediatric departments, which document social status, all children and adolescents <20 years of age with diabetes onset in 2002 to 2005 and with at least one in- or outpatient contact and documentation of the social status, were included (n=1,127, 55% male, age at onset (SD) 8.5 (4.2) years, mean follow up 1.2 years (SD 0.9, range 0.2–3.7 years)). We evaluated the association between four social parameters (parental school education and professional training, family situation, nationality) and the risk for post-onset hospital admission as well as number of hospital days. We estimated relative risks with 95% confidence intervals (RR, CI), adjusting for

age and sex. To take the cluster effect of centres into account, we used random effects models.

Results: 42% of the study subjects were hospitalized at least once during the follow up period. 50% of hospital admissions were due to education. Incidence of hospital admissions was 0.47 (0.43–0.50) per person-year (py), hospital days 2.78 (2.70–2.86) per py. Hospital admissions and hospital days were significantly higher in children from single parent families (RR 1.57; 95% CI 1.31–1.89 and 1.63; 1.51–1.76) and from lower school and professionally educated parents (RR's between 1.45 and 2.34, all $p<0.05$). In children from non-German and German families, the differences in hospital days were smaller (RR 1.25; 1.15–1.35), and there was no significant difference with respect to hospital admissions (RR 1.15; 0.94–1.39).

Conclusion: As expected, hospitalization was higher in diabetic children and adolescents from lower educated parents and incomplete families, indicators for low social status. Interestingly, there were less differences between German and non-German children. This finding may be explained by different patterns of health care seeking, maybe by lower participation in structured diabetes education in non-German families. The observations need to be confirmed and explained.

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0180

A comparison of diabetes-related quality of life between adolescents with type 1 diabetes and their parents

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Backround and Aims: As part of a randomized 24-week clinical trial in which insulin glargine was compared to twice-daily intermediate-acting insulin, diabetes-related quality of life (QOL) among adolescents with type 1 diabetes (T1DM) and their parents was evaluated at baseline.

Materials and Methods: The inclusion criteria for the study were males and females aged 9 to 17 years, Tanner stage≥2, with a diagnosis of T1DM for at least 1 year that was treated only with insulin. QOL was measured using the Youth and Caregiver modified versions of the Diabetes Quality-of-Life Measure, a self-administered questionnaire in which subjects responded to questions within 3 domains (diabetes impact [DI], diabetes-related worries [DW], and life satisfaction [LS]) using a 5-point Likert scale where higher scores represent poorer QOL. We limited our analysis to domain items that were similar in both the youth and parent questionnaires in order to facilitate direct comparisons. The data were analyzed using mixed-effects models with domain score as the dependent variable and youths' gender, respondent (parent or youth), study site, and gender-by-respondent interaction as independent variables.

Results: A total of 175 adolescents (80 male, 95 female) were enrolled with a mean baseline A1C of 7.9%.

The female parent was the respondent in 86% of the surveys. Adjusted mean scores at baseline were higher (worse) for parents than children in the DI, DW, and LS domains, these differences were more pronounced when the child was male (Table).

Adjusted Mean Domain Scores by Gender and Respondent

Domain/Child's Gender	Parents	Youths	*P* Value
DI			
–Overall	23.2	20.6	<0.0001
–Female	23.0	21.4	.0216
–Male	23.5	19.7	<0.0001
DW			
–Overall	22.7	15.9	<0.0001
–Female	23.0	16.9	<0.0001
–Male	22.5	14.8	<0.0001
LS			
–Overall	27.6	26.9	.2306
–Female	27.4	28.0	.4341
–Male	27.9	25.7	.0199

Conclusion: We conclude that T1DM has a greater perceived effect on the QOL of parents than on the children themselves in terms of diabetes impact and diabetes–related worries, the parent–child difference being greater when the child is male.

Supported by sanofi–aventis U.S.

OP 31

Inhaled insulin

0181

Superior post prandial glucose control in patients with type 1 diabetes when using prandial technosphere insulin compared to NovoLog

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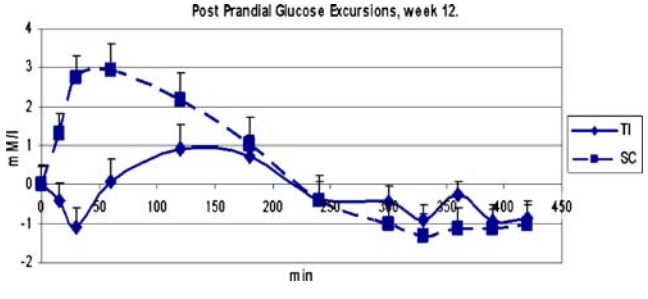
Background and Aims: A major challenge in basal-prandial insulin therapy is to achieve appropriate synchronization between meal absorption and prandial insulin activity. Technosphere insulin (TI) has an action profile with a faster onset and shorter duration of action than seen with existing insulin therapy, and might be ideally suited

for prandial insulin substitution. This study evaluated post-prandial glucose excursions following a standard liquid meal and overall glycemic control in a randomized controlled study of patients with type 1 diabetes.

Materials and Methods: 110 patients with type 1 diabetes (41 male and 69 female) already using a basal-prandial regimen were randomized to receiving TI (N=54) or insulin aspart, a rapid acting insulin analogue (RAA), (N=56) at meal times together with insulin glargine as basal insulin. After a transfer period, patients where followed for 3 month during which individual adjustments of therapy was performed, and standardized meal test (Boost Plus) were conducted at 0, 8 and 12 weeks of therapy.

Results: The study population (all Caucasian) had a mean age of 34 years, and a BMI of 24.4 kg/m^2, with no statistical difference between groups. Similar reductions inHbA1c where seen in both groups. The reduction from baseline was 0.83 (1.11) (mean (SD); ($p<0.001$) in the TI group and 0.99 (1.07); ($p<0.001$) in the group receiving sc RAA, with no statistical difference between groups; ($p=0.458$). At the same time, body weight decreased by 0.41 (2.22) kg in the TI group, while it increased by 0.89 (1.92) kg in the group receiving SC insulin. The difference in weight change between the groups was statistically significant ($p=0.0016$). An improvement in post-prandial blood glucose excursions was observed in subjects receiving TI as compared to RAA. Maximal post-prandial excursion at visit 10 was 0.92 mmol/L for TI vs. 3.0 mmol/L for RAA. The total post-prandial glucose elevation (AUCgluc) was 96.7 mmol/L*min for TI and 400.6 mmol/L*min for RAA. No changes in lung function was seen after 3 months treatment, (change in FEV1 was −0.064 l (0.189) for TI and −0.072 (0.193) for RAA ($p=0.82$; n.s.), and for DLco −1.62 (3.29) and −1.094 (3.08) ($p=0.39$; n.s.) respectively.

Conclusion: Used in a basal-prandial regimen, post prandial excursions where significantly less when using TI compared to RAA. TI provided similar improvement in glycemic control (expressed as change from baseline HbA1c) to RAA, without an increase in body weight. No adverse effect was seen on pulmonary function.



Supported by MannKind Corporation

0182

Impact of exercise on absorption of inhaled insulin

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Background and Aims: Inhaled insulin is a new alternative to injection of insulin. Little is however known about the effect of exercise on the absorption of inhaled insulin. Thus, the purpose of this trial was to investigate the effect of moderate exercise on the absorption of inhaled human insulin in administered by a commercial nebuliser.

Materials and Methods: This single-center, open-labeled, three-period cross-over trial included a total 12 non-smoking healthy subjects (28.6±4.9 years (mean±SD), BMI 23.2±2.7 kg/m^2). On three different dosing days, in randomised order, the subjects received a dose of 3.5 mg fast-acting inhaled human insulin administered by a commercial nebuliser (MedicAidpro-JAEGER APS) followed by either 1) No Exercise, 2) 30 min moderate exercise starting immediately after dosing ($Exercise_0$), or 3) 30 min moderate exercise starting 30 min after dosing ($Exercise_{30}$). The study was carried out as a 10 hour euglycemic glucose clamp (90 mg/dL (5.0 mM)).

Results: Area under the serum insulin curve (AUC_{ins}) from 0 to 120 minutes after start of exercise was 16% increased for $Exercise_0$ (Ratio[95%CI] for $AUC_{ins(0–120min)}$:1.16 [1.04;1.30], $p=0.01$) and 20% increased for $Exercise_{30}$ ($AUC_{ins(30–150min)}$:1.20 [1.05;1.36], $p<0.01$), both compared to No Exercise. The effect of exercise on the overall insulin absorption during the first 6 hours ($AUC_{ins(0–360\ min)}$), and the complete 10 hours ($AUC_{ins(0–600\ min)}$) after dosing was not statistically significant. The maximum insulin concentration (C_{max}) was increased by 32% for $Exercise_0$ and 35% for $Exercise_{30}$ (both $p<0.01$) compared to No Exercise, while the time to C_{max} (t_{max}) was 31 min faster for $Exercise_0$ ($p<0.01$), but not significantly different after $Exercise_{30}$ compared to No Exercise. The appearance of insulin in plasma was faster after exercise compared to No Exercise, with an initial rate of increase (best fitted line from baseline to t_{max}) 150% larger for $Exercise_0$ ($p<0.01$) and 72% larger for $Exercise_{30}$ ($p=0.02$). There was no effect of exercise on the terminal elimination rate constant.

The integrated glucose infusion rate (AUC_{GIR}) for 2 hours after start of exercise increased by 95% for $Exercise_0$ and 123% for $Exercise_{30}$ (both $p<0.01$) compared to No Exercise, whereas AUC_{GIR} for the first 6 hours, and

complete 10 hours after exercise was not statistically significant different. Compared to No Exercise, the maximal GIR (GIR_{max}) was increased by 59% for $Exercise_0$ ($p=0.02$) and 93% for $Exercise_{30}$ ($p<0.01$), and occurred 66 min faster for $Exercise_0$ ($p<0.01$) and 38 min faster for $Exercise_{30}$ ($p=0.02$).No safety issues or adverse events related to the inhaled human insulin were observed.

Conclusion: Thirty minutes of moderate exercise immediately or 30 minutes after dosing of inhaled human insulin leads to increased absorption, with higher C_{max} over the first 2 hours after start of exercise, and a shorter t_{max} for exercise immediately after dosing. These data indicate that patients using inhaled insulin should expect an increased absorption of insulin in connection with exercise shortly after inhaling insulin, but not in the overall absorption.

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0183

Effects of albuterol and fluticasone on Inhaled Human Insulin (Exubera®) pharmacokinetics in subjects with mild-to-moderate asthma

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Background and Aims: Inhaled asthma treatments and inhaled human insulin (INH; Exubera® (insulin human [rDNA origin]) Inhalation Powder) share a common route of administration, therefore it is important to characterize the potential effects of concomitant administration. This study assessed the effects of prior administration of albuterol (ALB, a short-acting β-agonist) and fluticasone (FLU, an inhaled corticosteroid) on INH pharmacokinetics.

Materials and Methods: This open-label, crossover study randomized 67 nondiabetic subjects with asthma (mean unmedicated forced expiratory volume [FEV_1]≥80%–100% [mild] or≥50%–<80% [moderate] of predicted values) to INH (3 mg) alone, ALB (180 μg)+INH (3 mg) 30 minutes later, INH 3 mg then ALB 180 μg 10 minutes later, or FLU (440 μg)+INH (3 mg) 30 minutes later. Healthy nonasthmatic volunteers received INH (3 mg).

Results: Prior ALB administration increased mean maximum insulin concentration (C_{max}) and area under the curve ($AUC_{0–360}$) by ~25% to 35% in patients with mild asthma and by ~45% to 50% in patients with moderate asthma relative to INH administration without ALB (Table 1). The increased INH bioavailability following ALB administration resulted in mean AUC and C_{max} values that approximated those in healthy subjects without asthma (data not shown). Prior FLU administration had no significant effect on INH pharmacokinetics; point estimates of ratios for AUC and C_{max} ranged from 94.3% to 98.8% and all 95% CIs included 100% (Table 2). In asthmatic subjects, there were no remarkable differences in frequency or severity of adverse events between treatment groups.

Conclusion: In the absence of a bronchodilator, mild-to-moderate asthma was associated with reduced pulmonary absorption of INH. Administration of a bronchodilator 30 minutes before INH in subjects with mild-to-moderate asthma resulted in INH bioavailability that approximated bioavailability in nonasthmatic subjects. Therefore, to avoid undue variability in exposure, it is recommended that the timing of such administration to be consistent. These results suggest INH and fluticasone can be administered concomitantly without impacting insulin levels.

Table 1

	Moderate asthma			Mild asthma		
	ALB+ INH*	INH Alone*	Ratio (%) (95% CI)	ALB +INH*	INH Alone*	Ratio (%) (95% CI)
Insulin $AUC_{0–360}$ (μU.min/mL)	2941	1953	150.6 (119.8, 89.4)	5052	4056	124.6 (106.2, 146.2)
Insulin C_{max} (μU/mL)	29.6	20.1	147.0 (120.2, 179.8)	46.8	34.6	135.2 (115.1, 158.7)

*Adjusted geometric means.CI=confidence interval.

Table 2

	Moderate Asthma			Mild Asthma		
	FLU +INH*	INH Alone*	Ratio (%) (95% CI)	FLU +INH*	INH Alone*	Ratio (%) (95% CI)
Insulin $AUC_{0–360}$ (μU.min/mL)	1843	1953	94.3 (74.9–118.8)	4009	4056	98.8 (83.9–116.4)
Insulin C_{max} (μU/mL)	19.1	20.1	94.8 (77.4–116.1)	34.2	34.6	98.8 (83.8–116.4)

*Adjusted geometric means

Supported by: Pfizer Inc

0184

Human insulin inhalation powder (HIIP) in subjects with chronic obstructive pulmonary disease (COPD): pharmacokinetic (PK) and glucodynamic (GD) responses

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Background and Aims: Only limited information is available on the PK and GD effects of inhaled insulin in patients with COPD. This open-label, randomized, 3-period crossover trial compared PK and GD responses in healthy subjects and in subjects with COPD after administration of HIIP (Lilly/Alkermes inhaled insulin system) versus administration of subcutaneous (SC) insulin lispro.

Materials and Methods: Nonsmoking subjects, 15 healthy (age 38±13 years) and 30 with moderate COPD (IIA or IIB per Global Initiative for Chronic Obstructive Lung Disease criteria), 15 each with chronic bronchitis (53±9 years) and emphysaema (58±6 years) received 2 single doses of HIIP (5.2 mg each) and 1 dose of SC insulin lispro (12 U). PK parameters were assessed by analyzing the serum insulin concentration data and GD parameters were derived from the euglycaemic glucose clamp data. Safety was assessed by laboratory pulmonary function tests (PFTs) before and after each clamp and by spirometry performed 4 times during clamps.

Results: HIIP and insulin lispro were absorbed rapidly in all 3 subject groups (median t_{max} 45–60 min). Total insulin exposure ($AUC_{(0–t')}$) and metabolic effect (GIR_{tot}) after SC insulin lispro were comparable in all 3 groups. Insulin exposure ($AUC_{(0–t')}$) after HIIP administration was reduced by 22% ($p=0.13$) in subjects with emphysaema and by 44% ($p<0.001$) in those with chronic bronchitis as compared to healthy subjects. Accordingly, GIR_{tot} after HIIP was reduced in subjects with emphysema (33%, $p<0.01$) and chronic bronchitis (40%, $p<0.01$). Intra-subject variability for HIIP exposure, based on PK $AUC_{(0–t')}$ was comparable between healthy subjects and those with emphysaema (29 and 28% CV), but was greater in subjects with chronic bronchitis (52% CV). No statistically significant differences between the pre- and post-clamp PFTs were observed. During clamps, FEV1 and FVC declined modestly in both COPD groups, with no difference between HIIP and SC insulin lispro treatments.

Conclusion: The Lilly/Alkermes HIIP system was well tolerated by COPD subjects, showing similar time-exposure and time-action profiles but reduced overall exposure and effect compared to healthy subjects. Long-term safety evaluation of HIIP in patients with COPD and diabetes is warranted.

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0185

Technosphere insulin does not affect pulmonary function in a 6 month study of patients with type 2 diabetes

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Background and Aims: The prospect of replacing prandial insulin injections with insulin inhalation is attractive to many physicians and patients. However, changes in pulmonary function have been reported with other formulations of pulmonary insulin, and this study was undertaken to evaluate the safety of Technosphere insulin (TI) in this respect.

Materials and Methods: 306 patients with type 2 diabetes with HbA1c levels of 7–11.5% were enrolled in a randomized, controlled, 6 month study. Prior to enrolling, patients had been treated with sc insulin for at least 3 month. At entry, patients initiated treatment with insulin glargine. Following a 3 week titration period, patients were randomized to either TI or insulin aspart at meal times. Pulmonary function testing, including FEV1, FVC and FEF 25–75%, was conducted at entry, randomization and at 2 and 6 month. All pulmonary laboratories were uniformly trained and monitored and procedures were standardized according to American Thoracic Society recommendations.

Results: The study population (n=306) had a mean age of 58 years, and a BMI of 30.8 kg/m^2, with no statistical difference between groups. The TI group consisted of 148 subjects (39 male/109 female) and the control group of 158 subjects (33 male/125 female). The results of pulmonary function testing are listed in the table. For all parameters, the differences were not statistically significant. Pulmonary function is susceptible to changes in age and weight. When a correction for these parameters was applied, (using the algorithm of A Miller et al), no numeric change from baseline was observed.

Pulmonary Function Measurements

L mean (SD)	FEV1 TI	FEV1 SC	FVC TI	FVC SC	FEF25–75% TI	FEF25–75% SC
Week -4	2.559	2.501	3.282	3.237	2.362	2.273
	(0.71)	(0.54)	(0.88)	(0.70)	(1.00)	(0.88)
Week 0	2.540	2.476	3.266	3.196	2.330	2.281
	(0.71)	(0.53)	(0.88)	(0.67)	(1.0)	(0.93)
Week 8	2.545	2.471	3.287	3.198	2.276	2.257
	(0.74)	(0.53)	(0.89)	(0.67)	(1.04)	(0.94)
Week 12	2.542	2.433	3.29	3.152	2.298	2.190
	(0.76)	(0.53)	(0.93)	(0.64)	(1.6)	(0.92)

Conclusion: There was no change pulmonary function after 6 month treatment with TI compared with treatment with sc insulin.

Supported by MannKind Corporation

0186

Sustained efficacy and tolerability of inhaled human insulin (Exubera®). Therapy over 2 years: patients with type 1 diabetes

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Background and Aims: The availability of inhaled human insulin (INH; Exubera® (insulin human [rDNA origin]) Inhalation Powder) may increase patient acceptance of insulin, improve glycemic control, and reduce the burden of diabetes. Two previous 12- and 24-week studies of INH versus subcutaneous (SC) insulin in patients with type 1 diabetes mellitus (T1DM) showed comparable efficacy and small treatment group differences in lung function (measured using routine pulmonary function testing [PFT]), but long-term studies are critical to better characterize pulmonary safety. The aim of this study was to investigate the long-term (2-year) efficacy and safety profile of INH therapy in patients with T1DM.

Materials and Methods: An open-label, parallel-group, multicenter study where patients (between 18 and 65 years of age) were randomized to INH (n=291) or SC insulin (n=291). The primary end point was pulmonary function, as measured using highly standardized PFT. Secondary end points included glycosylated hemoglobin (HbA_{1c}), hypoglycemia, fasting plasma glucose (FPG), and body weight.

Results: Treatment group differences in changes from baseline lung function (forced expiratory volume in 1 second [FEV_1] and carbon monoxide diffusing capacity [DL_{co}]) were small (<1% and<2% of mean baseline values, respectively), occurred early (Month 3 data: −0.021 L [90% confidence interval [CI], −0.041, −0.002] for FEV_1 and −0.687 mL/min/mm Hg [−0.969, −0.406] for DL_{co}), and remained stable with no progression for up to 2 years (−0.023 [−0.044, −0.002] and −0.439 [−0.732, −0.145], for FEV_1 and DL_{co}, respectively). Glycemic control was maintained in both groups (change from baseline to Month 24 in HbA_{1c} was 0.1% and −0.2% for INH and SC groups, respectively). The incidence of hypoglycemic events was similar in both groups (4.0 vs 3.8 events/subject–month, for INH and SC, respectively), but the incidence of severe hypoglycemic events was lower with INH (2.8 events/100 subject–month) versus SC (4.1 events/100 subject–month). After 24 months, INH resulted in greater reductions in FPG compared with SC insulin (from 9.5 to 8.7, vs 9.3 to 9.6 mmol/L, [170.1 to 156.8, vs 166.9 to 173.5 mg/dL], respectively), and less body weight gain (mean 0.8±4.2 kg vs 2.0±4.6 kg). Adverse events were generally comparable with the exception of cough in the INH group, which was mild in most patients, nonproductive, and closely associated with dosing.

Conclusion: This 2-year study shows that INH is well tolerated and provides comparable glycemic control to SC insulin in adult patients with type 1 diabetes.

Supported by Pfizer Inc

OP 32

Neuropathy

0187

Influence of blood glucose oscillations, measured by continuous glucose monitoring, on QTc interval and sympatho-vagal balance, during the day-life of type 1 diabetic patients

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Background and Aims: Several recent studies have described a relationship between QTc prolongation and increased mortality in type 1 diabetic patients. Both hypoglycaemia and hyperglycaemia have shown to acutely increase QTc duration and stimulate sympathetic activation. However, only few, preliminary data are available on the role of blood glucose modifications on cardiac repolarization and sympatho-vagal balance, under physiological condition. Aim of the present study was to investigate the influence of blood glucose oscillations, during the day-life

of type 1 diabetic patients, on QTc duration and on sympatho-vagal balance.

Materials and Methods: 22 type 1 diabetic patients (11 males and 11 females) were studied. Patients were in good metabolic control, mean age 34.4+9.9 years; exclusion criteria were hypertension, medication known to modify QT and diabetic complications. A 24-h electrocardiogram was recorded in each patient, with automatic QTc and R–R measurement, for the evaluation of sympatho-vagal balance (LF/HF ratio). Simultaneously, interstitial glucose concentrations were measured via a subcutaneous abdominal catheter using a continuous glucose monitoring system. Mean hourly values of blood glucose were divided into quintiles: I.<60; II. >60<100; III. >100<140; IV. >140<190; V. >190 mg/dl.

Results: A significant increase of QTc interval was observed in the V quintile vs. II and III (436+19.4 vs. 423.4+22.5 and 425.4+24.6 ms, respectively, $p<0.001$), with a significant correlation between blood glucose and QTc in the V quintile, thus suggesting a role of hyperglycemia on QTc lengthening. LF/HF ratio was significantly increased in the I vs. the II and the III quintile (2.2+1.5 vs. 2.7+1.7 and vs. 2.8+1.9, respectively, $p<0.03$) and in the V vs. the II and the III quintile ($p<0.04$); in the V quintile, LF/HF significantly correlated with QTc ($p=0.005$).

Conclusion: In conclusion, hyperglycemia seems to represent the most important factor in influencing QTc interval increase and sympathetic activation in well controlled, type 1 diabetic patients, without diabetic complications.

0188

Preservation of thalamic neuronal function may be a prerequisite for pain perception in diabetic neuropathy

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Background: Pain is the most distressing symptom of diabetic neuropathy (DN). Despite extensive research, the pathogenesis of pain in DN remains poorly understood. As a result, treatment of painful DN is frequently unsatisfactory. The thalamus, which is the gateway to the somtosensory cortex, may have a role in the pathogenesis and persistence of painful DN.

Methods: 23 subjects with diabetes (7 No-DN, 8 painless DN and 8 painful DN) and 6 healthy controls (HC) underwent detailed clinical and neurophysiological assessments (Dyck's staging criteria; NIS(LL)+7). Proton magnetic resonance spectroscopy of the right postero-lateral thalamic nucleus was performed to measure established markers (N-acetyl [NA] compounds) of neuronal function using long echo time (LTE) and neuronal integrity using short echo time (STE) sequences.

Results: At LTE (ANOVA $p=0.003$), subjects with painless DN (mean=1.56 [SD=0.18]) had lower NA compared to painful DN (1.93[0.23] $p<0.001$). There was no significant difference between painful DN and the other groups (vs No-DN (1.78[0.15], $p=0.11$; vs HC (1.74[0.11], $p=0.052$). There were no significant differences in STE between the groups.

Conclusions: As NA is largely confined to neuronal cell bodies and axons, the significant difference between painless and painful DN is suggestive of a higher degree of thalamic dysfunction in painless DN and preserved thalamic sensory neuronal function in the latter. The lack of a difference in STE between the groups suggests no neuronal loss. Preservation of thalamic neuronal function may therefore be a prerequisite for the perception of pain.

Supported by Diabetes UK

0189

Effects of venlafaxine and carbamazepine for painful peripheral diabetic neuropathy: a randomized controlled double-blind and double-dummy multicenter clinical trial

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Background and Aims: Diabetic neuropathies are chronic complications of diabetes mellitus.Venlafaxine is a unique antidepressant with dual mechanisms of action. Several studies suggested that venlafaxine may benefit the patients with severe painful diabetic neuropathy. Venlafaxine extended release appeared effictive and safe in relieving painful diabetic neuropathy.Carbamazepine is an iminostilbene derivative chemically related to the tricyclic antidepressants.The studies showed that it can improve the symptoms of painful diabetic neuropathy.

Aim:To evaluate the safety and efficacy of venlafaxine and carbamazepine on painful peripheral diabetic neuropathy.

Materials and Methods: This clinical trial was a randomized, parallel-group, double-blind, double-dummy study. 132 Patients (venlafaxine group (n=66) and carbamazepine group (n=66)) with painful peripheral diabetic neuropathy

were recruited from 3 clinical centers. Venlafaxine group took venlafaxine 25 mg plus one dummy carbamazepine tablet twice times a day and carbamazepine group took carbamazepine 0.1 g plus one dummy venlafaxine tablet twice times a day for 2 weeks. The primary efficacy parameter was a numeric pain intensity scale and the secondary was to assess quality of life.

Results: 119 patients have completed the trial. Venlafaxine was superior to carbamazepine in improving mean pain intensity score at 5,7,10 and 14 days by PP analysis (p=0.0229, p=0.0256, p=0.0028 and p=0.0005 respectively). The effects of venlafaxine on improvement of the totle scores of quality of life were better than that of carbamazepine at 10 and 14 days (p=0.0183 and p=0.0037 respectively). Sleep interference and mood were improved by venlafaxine and carbamazepine, and the efficacy of venlafaxine was superior to that of carbamazepine. The common venlafaxine's adverse events included mild gastrointestinal discomfort, dizziness and somnolence. The frequency of adverse events in venlafaxine group was about 43.9% (4 patients withdrew for adverse events) and in carbamazepine group about 25.76% (2 patients withdrew for adverse events) (p=0.0558).

Conclusion: Venlafaxine and carbamazepine are efficacious in the treatment of painful diabetic neuropathy, venlafaxine is superior to carbamazepine in improving pain and quality of life. Both two drugs are safe and well tolerated.

0190

Safety and efficacy of CNS 5161, a new neuronal glutamate receptor antagonist (NMDA) in the treatment of painful peripheral neuropathy

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Background and Aims: Treatment of chronic pain syndromes associated with peripheral nerve injury is often a challenge. NMDA antagonists attenuate the frequency-dependent potentiation of dorsal horn nociceptive neurons and have been found to exert beneficial pain relief in patients with peripheral neuropathies.

Materials and Methods: This study was a double blind, dose escalating, crossover study evaluating the safety and efficacy of the new NMDA antagonist CNS 5161 compared to placebo. Forty patients with chronic pain syndromes; post herpetic pain, diabetic neuropathy, post traumatic nerve injury or chronic regional pain syndrome I or II; (23 male, 17 female, age 55.2±11.5 years, weight 81.9±17.9 kg (mean±SD)) were recruited. Patients were treated with one of four different dosages of CNS 5161 (125, 250, 500 and 750 μg). The patients received the study medication as a bolus intravenous infusion of 30% over 10 minutes and as a subsequent infusion of 70% over 5 hours 50 minutes. The incidence, severity and relationship to study drug of all adverse events were determined. Blood pressure, heart rate, ECG and clinical laboratory values were assessed. Pain relief was measured by visual analogue scales (VAS) and ordinal verbal pain scores at baseline and at 2, 6, 12 and 24 hours after study drug administration.

Results: Infusion with CNS 5161 at all dosages appeared to be associated with an increase in mean systolic blood pressure and to a lesser extent in diastolic blood pressure. Moderate blood pressure elevations were observed in 8.3% of patients receiving 250 μg and in 50% of patients receiving 500 μg CNS 5161, compared with 15.4% during placebo treatment. Only two patients were recruited for treatment with 750 μg CNS 5161 due to the blood pressure effects with increasing dosage. The most commonly occurring nervous system disorder was headache, which was found more often during placebo than during CNS 5161 treatment. Blurred vision was experienced by 16.7% receiving 250 μg and by 33.3% receiving 500 μg CNS 5161, but not during placebo treatment. Post treatment VAS revealed a tendency for better pain improvement after 500 μg CNS 5161 compared to placebo (1.16±2.15 vs. 0.23±1.87; p=0.11). No difference in the verbal pain scores could be observed between CNS 5161 and placebo treatment. The mean improvement in VAS was considerable higher in patients with diabetic neuropathy compared with all other nerve disorders: 1.7, 2.5 and 2.3 cm at 2, 6 and 12 hours respectively. In addition, the effect of CNS 5161 on verbal pain intensity and verbal pain relief seemed to be stronger in diabetic patients than in the other nerve disorders.

Conclusion: The new NMDA antagonist CNS 5161 was shown to be sufficiently well tolerated when used for treatment of neuropathic pain, with the most common side effects being a moderate increase in blood pressure and mild visual disturbances. Although this study was not powered for efficacy evaluation, treatment with CNS 5161 appeared to exert analgesic effects, especially in patients with diabetic neuropathy. Further sufficiently powered studies will investigate the clinical effects of CNS 5161 in this special patient population.

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0191

Transcutaneous electrical nerve stimulation (TENS) in diabetic neuropathy

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Background and Aims: Painful neuropathy is a common, and difficult to manage, complication of diabetes.There is a need for effective, efficient and safe methods of pain relief in these patients. This paper reviews the effect of TENS on different symptoms of painful neuropathy in patients of diabetes mellitus.

Materials and Methods: A single blind, randomized, prospective single-centre study was planned to compare TENS with oxcarbamazepine in the management of painful diabetic neuropathy. 180 adult diabetic patients receiving five sittings of TENS on daily or alternate day basis were compared with 125 age-matched, disease-matched patients who were given daily oxcarbamazepine and five sittings with sham electrodes.

Results: Pain scores reduced from 4.40±0.54 to 3.60±0.54 in the oxcarbamazepine group while they fell from 4.40±0.54 to 1.60±0.54 in the TENS group($p<0.05$). This difference was maintained after 3 week, even though the TENS sittings had stopped. Best reductions in pain score were achieved in subjects with burning (3.28±0.64) and lancinating (3.12±0.64). Least improvement was seen in allodynia (2.15±0.35) and restless legs syndrome (2.16±0.56)

A significant improvement was seen in health distress and disease intrusion scores in the TENS group

Conclusion: This study demonstrates the beneficial effects of low dose TENS in patients with painful diabetic neuropathy

0192

Gene transfer of an engineered transcription factor promoting expression of VEGF-A protects against experimental diabetic neuropathy

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Background and Aims: We investigated whether gene transfer of an engineered zinc finger protein transcription factor (ZFP TF) designed to upregulate expression of the endogenous vascular endothelial growth factor (VEGF)-A gene could protect against experimental diabetic neuropathy (DN). ZFP TF-driven activation of the endogenous gene results in expression of all of the VEGF-A isoforms.

Materials and Methods: Rats were made diabetic with streptozotocin (55 mg/kg i.p.) after an overnight fast and group-housed with food and water ad libitum. They were then maintained for 8-week protocols at the end of which motor and sensory nerve conduction velocities (MNCV, SNCV) were measured in both hind–limbs under isoflurane anaesthesia. Different groups were given intramuscular injections of a plasmid encoding the ZFP TF in the left hind–limb only; the right limb was not injected. Three diabetic groups were given this treatment at 2 or 4 or 6 weeks after induction of diabetes; another diabetic group was given this treatment at all these time points and a final diabetic group was injected at the three time point with a placebo plasmid that did not encode the ZFP TF (sham-injected group). A non-diabetic group formed the controls.

Results: The controls and sham injected group showed similar NCVs in both limbs, with the sham-injected diabetic rats showing falls of 19.1% (MNCV) and 21.4% (SNCV; both $p<0.001$) compared to controls. The diabetic rats injected 3 times with active plasmid showed reductions in NCV in the non-injected (right) limb of 17.0% (MNCV) and 25.0% (SNCV), with no significant reductions in NCV on the injected side and a marked statistically significant difference between the two sides ($p=0.011$ for MNCV and 0.002 for SNCV). The diabetic group injected with active plasmid at week 6 only (2 weeks before NCV measurement) showed similar data to the group receiving three injections (left–right comparisons were $p=0.066$ for MNCV and 0.005 for SNCV). There was a residual effect in the group injected at week 4, but the group injected at week 2 showed data similar to the sham-injected animals.

Conclusion: These data suggest that VEGF-A-activating ZFP TFs may ultimately be of clinical utility in the treatment of diabetic neuropathy.

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OP 33

Mitochondrial dysfunction and insulin resistance

0193

Mitochondrial respiratory function is decreased in skeletal muscles of patients with type 2 diabetes

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Aim: This study was undertaken to investigate whether decreased oxidative capacity in type 2 diabetic (T2D) patients is caused by decreased mitochondrial volume or impaired mitochondrial functionality.
Methods: Skeletal muscle biopsies obtained from 10 obese T2D patients and 8 obese control subjects were used for assessment of 3-hydroxy-Acyl-CoA-dehydrogenase (HAD) and citrate synthase (CS) activity, uncoupling protein 3 (UCP3) content, fiber type distribution and respiration in isolated mitochondria using different substrates. To account for mitochondrial volume measures of respiration were corrected for CS activity.
Results: T2D patients had a significant lower state 3 respiration compared to obese controls using pyruvate+ malate but not when using palmitoyl-L-carnitine+malate. Furthermore, maximal activity of the electron transport chain was lower in T2D patients. No differences in CS or HAD, or overall respiration per mg muscle protein were observed between T2D patients and obese controls. T2D patients had a higher percentage of type 2b fibers whereas no differences in percentage type 1 fibers or UCP3 content were observed. Correlation analysis in whole population (n=18) revealed a significant negative correlation between state 3 using pyruvate+malate and HbA1c. State 4 respiration (regardless of substrate) was positively correlated to percentage of type 2b fibers. Furthermore, percentage type 2b fibers correlated positively with several markers of insulin resistance.
Conclusion: In conclusion we provide evidence for a functional impairment in mitochondrial respiration and increased amount of type 2b fibers in skeletal muscle of patients with T2D. These alterations may be important factors contributing to the progression from obesity to type 2 diabetes.
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0194

Muscle mitochondrial dysfunction with age-impact of body composition and insulin sensitivity

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Background and Aims: Muscle mitochondrial dysfunction has been reported in aging, type 2 diabetes mellitus, obesity and other insulin resistant states. We sought to determine whether muscle mitochondrial dysfunction in aging is related to change in body composition, insulin resistance or age *per se.*
Materials and Methods: We studied 12 each of elderly lean (EL, BMI=22.6±0.6 kg/m^2), elderly obese (EO, BMI=30.0±0.6), young lean (YL, BMI=23.0±0.5) and young obese (YO, BMI=30.9±0.6) who have similar leisure time activity levels and fat free mass. Hyperinsulinemic (1.5 mU/kg FFM/min) euglycemic clamp to assess insulin sensitivity was performed. Freshly isolated mitochondria (mt) from needle vastus lateralis muscle biopsy samples was used to measure ATP production based on luciferase reaction.
Results: Elderly people irrespective of their differences in BMI had lower VO_2 peak (32.42±1.26 ml/kgFFM/min) than in younger people (47.58±0.94 ml/kgFFM/min, p<0.0001). mtATP production using pyruvate, palmitoyl-L-carnitine, ketoglutarate and malate (PPKM) was higher (5.51±0.2 μmol/min/g tissue) in younger people than in older people (3.97±0.33, p=0.0003) and there were no differences between obese and lean groups. Glucose infusion rate (GIR) was higher in lean people (54.28±2.41 mmol/kgFFM/min) than in obese group (42.7±2.46, p=0.002) to maintain similar glucose levels at steady state (lean −90.9±0.06 mg/dL vs obese −90.9±0.04, p=NS). There was no effect of age on GIR. Insulin sensitivity had no effect on mtATP production or vice versa.
Conclusion: In conclusion, obesity is the major determinant of insulin sensitivity, while age and mtATP production show no significant effect. However, the decline in muscle mitochondrial ATP production with age is unrelated to the differences in obesity and insulin sensitivity.
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0195

Impaired *in vivo* mitochondrial function but similar intramyocellular lipid content in patients with type 2 diabetes mellitus compared to BMI–matched controls

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Background and Aims: Mitochondrial dysfunction and increased IMCL content have both been implicated in the development of type 2 diabetes mellitus (T2DM), but the relative contribution of these two factors in the aetiology of diabetes is unknown. As obesity is an independent determinant of IMCL content, we here examined mitochondrial function and IMCL content in overweight type 2 diabetic patients and BMI matched controls.
Materials and Methods: Twelve patients with T2DM and nine BMI matched normoglycemic controls were investigated (age (y): 61.8±4.4 and 56.1±6.8, $p<0.05$; BMI (kg/m^2): 29.4±3.3and 29.3±2.7, p=n.s.; VO_2max ($ml*min^{-1}*kg^{-1}$): 30.6±5.8 and 34.6±4.8, p=n.s., in T2DM and controls, respectively (mean±stdev)). Mitochondrial function was determined by measuring PCr recovery half-time immediately after exercise with 31-phosphorus magnetic resonance spectroscopy (^{31}P-MRS). IMCL content was determined by proton magnetic resonance spectroscopic imaging (^{1}H-MRSI) and insulin sensitivity was assessed with a hyperinsulinemic euglycemic clamp.
Results: The half-time of PCr recovery was 45% longer in T2DM patients (27.3±3.5 s versus 18.7±0.9 s,), while IMCL content was similar as in controls (1.37±0.30% vs. 1.25±0.22% of the water resonance, p=n.s.). Insulin sensitivity was lower in T2DM patients (26.0±2.2 and 18.9±2.3 $\mu mol*min^{-1}*kg^{-1}$, (all mean±SEM), $p<0.05$). The recovery half-time of PCr correlated positively with plasma glucose ($R^2=0.42$, $p<0.01$) and HbA1c ($R^2=0.48$, $p<0.05$) in diabetic patients.
Conclusion: The finding that *in vivo* mitochondrial function is decreased in T2DM patients while IMCL content is similar as in controls suggests that a low mitochondrial function is a more important determinant than a high IMCL content in the aetiology of T2DM. The correlation of plasma glucose concentration as well as HbA1c with PCr recovery half-time reveals that the impairment of *in vivo* mitochondrial function is related to the degree of the disease. Our results are in line with the hypothesis that decreased mitochondrial function is a predisposition to develop type 2 diabetes mellitus.

0196

In vivo mitochondrial function in young men born with low birth weight, as assessed by ^{31}P-NMR spectroscopy after energy depleting exercise

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Background and Aims: Low birth weight (LBW) is associated with increased risk of insulin resistance and development of type 2 diabetes (T2D) later in life. The underlying molecular mechanism(s) remain largely undetermined. Recent magnetic resonance spectroscopy studies in humans suggest, that even subtle defects in mitochondrial function may play a role in the pathogenesis of (muscle) insulin resistance in aging and T2D. Mitochondrial dysfunction in the liver and pancreatic beta cells has also recently been linked with impaired insulin secretion in growth retarded rats, indicating a general intrauterine programming mechanism. The aim of this study was to determine whether LBW in humans is associated with impaired mitochondrial function in skeletal muscle tissue after energy depleting exercise.
Materials and Methods: We evaluated *in vivo* mitochondrial function in the forearm flexor muscle by ^{31}phosphorus nuclear magnetic resonance spectroscopy (^{31}P NMR) in 18 24-year old healthy lean men with LBW (BW ≤ 10th percentile) and 16 matched normal birth weight (NBW) (50th ≤ BW ≤ 90th percentile) controls. There was no difference between the groups with regards to BMI, W/H, fat mass and lean mass; however fasting b-glucose was higher in LBW subjects 4.59±0.43 vs. 4.99±0.48 mmol/L (P=0.003). A standard exercise protocol was performed with 4 successive maximal voluntary contractions each lasting 30 sec interspersed by a 60 sec recovery period. The ^{31}P NMR technique provides non-invasive real-time estimates of resting state parameters of energy metabolism as well as mitochondrial aerobic capacity.
Results: In the forearm flexor muscle, the recovery of PCr (doubling time) after exercise was increased by 35% in the LBW group, 57±19 vs. 76±28 sec. (P=0.05). There were no other differences between the NBW and LBW group with regards to resting PCr or PCr levels after exercise, pH at rest and after exercise or in the calculated Vmax for mitochondrial ATP synthesis. Insulin resistance as determined by using the euglycemic hyperinsulinemic clamp technique was not correlated with recovery of PCr or other measures of *in vivo* mitochondrial function.
Conclusion: The PCr recovery kinetics was altered in 24-year old healthy men with low birth weight, suggesting that the mitochondrial function is affected in this group. These data could imply that the altered mitochondrial function could possibly forego the development of an impaired glucose metabolism. Importantly, as these changes occur in young, lean non-diabetic subjects, we speculate that mitochondrial dysfunction may play a primary role in the pathogenesis of muscle insulin resistance and T2D associated with low birth weight.
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0197

Mitochondrial dysfunction is not due to impaired transmembrane glucose transport in type 2 diabetes

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Background: Type 2 diabetic patients (T2DM) and their relatives exhibit defective energy metabolism. However, factors determining mitochondrial dysfunction are yet unclear. We measured mitochondrial function noninvasively from ATP synthetic flux (fATP) and analyzed relationships with insulin resistance and transmembrane myocellular glucose transport in humans.
Materials and Methods: Well controlled T2DM (n=10; age:59±6a, nondiabetic older (CONo: n=10; 57±6a) and younger humans (CONy: n=11; 27±2a; $p<10^{-6}$ vs T2DM and CONo) were studied. Muscle glucose-6-phosphate (G6P), inorganic phosphate (Pi) and fATP were measured before (bfATP) and during hyperinsulinemic–euglycemic clamp (ifATP) using ^{31}P magnetic resonance spectroscopy (MRS). Four T2DM underwent a further hyperinsulinemic–hyperglycemic clamp. Insulin sensitivity was assessed from steady state glucose infusion rate (M). Intramyocellular lipid contents (IMCL) were quantified with ^{1}H MRS.
Results: M differed between T2DM, CONo and CONy (5.4±2, 8.3±1, 12.2±3 $mg.kg^{-1}.min^{-1}$; p<0.05), whereas IMCL were similar in all groups. BfATP was ~37% lower in T2DM than in CONy (8.6±2, vs 11.8±3 $\mu mol.g^{-1}.min^{-1}$; p<0.05). Insulin increased fATP by 26% and 9% only in CONy and CONo (p<0.05). Fasting G6P and Pi were comparable and increased by ~50% and ~17% during the clamp in all study groups (G6P: T2DM: 224±97, CONo: 225±45, CONy: 221±72; p<0.05 vs baseline; Pi: T2DM: 3.3±0.4, CONo: 3.6±0.5, CONy: 3.2±0.3; p<0.005 vs baseline). BfATP and ifATP related positively to M ($R \geq 0.40$, p<0.05) and negatively to basal plasma free fatty acids ($R \geq -0.45$, p<0.05). During hyperglycemic clamps, M was ~70% (8.1±2 vs 4.8±1 $mg.kg^{-1}$ body $weight.min^{-1}$, $P<0.05$) and G6P was ~100% higher than during normoglycemic clamps (290±1 vs. 145±5 $\mu g.kg\ muscle^{-1}$, p=0.01). BfATP and ifATP did not differ between normoglycemic and hyperglycemic clamps (bfATP: 9.0±2 vs 9.4±2, ifATP: 9.2±4 vs 10.3±2 $\mu mol.g^{-1}.min^{-1}$).
Conclusion: Muscle ATP synthesis reflects insulin sensitive glucose disposal and decreases with increased lipid availability. Hyperglycemia increases glucose transport but fails to improve the impairment of ATP synthesis in well-controlled T2DM indicating the presence of a mitochondrial defect independent of substrate flux.
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0198

Genetic, epigenetic and environmental factors influence oxidative phosphorylation genes and metabolism in human muscle

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Background and Aims: A set of genes involved in oxidative phosphorylation (OXPHOS) show reduced expression in muscle of patients with type 2 diabetes, but it remains unknown whether this is an inherited or acquired defect. Here we investigated whether genetic, epigenetic and non-genetic factors influence the expression of 4 OXPHOS genes, NDUFB6 (complex 1), UQCRB (complex 3), COX7A1 (complex 4) and ATP5O (complex 5) in human muscle.
Materials and Methods: Muscle biopsies were taken from young (n=86, age 28±0.2 years) and elderly (n=68, age 62.4±0.2 years) monozygotic (MZ) and dizygotic (DZ) healthy twins before and after a hyperinsulinemic euglycemic clamp.
Results: The mRNA expression of NDUFB6, UQCRB, COX7A1 and ATP5O was reduced by 15%–40% (P<0.007) in muscle biopsies from elderly compared with young twins. When a classical twin approach and a biometric model were used, we found that both genetic and environmental factors influence the expression of these four OXPHOS genes in muscle. Regression analysis were then performed to test if any of the following non-genetic factors: muscle PGC-1α and PGC-1β expression, birth weight, age, sex, percentage body fat, trunk–leg fat ratio and VO_2max, influence muscle OXPHOS expression. Indeed, NDUFB6 expression was positively related to PGC-1α levels ($P<0.0001$), trunk–leg fat ratio ($P<0.05$) and VO_2max ($P<0.007$). UQCRB expression was positively related to PGC-1α ($P<0.0001$), percentage body fat ($P<0.02$) and VO_2max ($P<0.001$). COX7A1 expression was positively related to PGC-1α ($P<0.0001$), PGC-1β levels ($P<0.02$) and percentage body fat ($P<0.04$), and related to age ($P<0.0001$) and sex ($P<0.0001$). ATP5O expression was positively related to PGC-1α ($P<0.0001$) and related to age ($P<0.0001$) and sex ($P<0.003$). We then

used regression analysis to test if any of the four OXPHOS genes influence muscle metabolism, and NDUFB6 was the only OXPHOS gene associated with insulin-stimulated glucose -uptake (P<0.05), -oxidation (P<0.008) and -storage (P<0.05) as well as lipid oxidation (P<0.03). We therefore focused our genetic and epigenetic analysis on *NDUFB6* and we identified a polymorphism (A/G) in the promoter-region of the *NDUFB6* gene, creating a possible transcription factor binding site and a DNA methylation site (a CG-dinucleotide). Interestingly, elderly carriers of the G/G genotype had reduced NDUFB6 expression compared with carriers of A/A and A/G genotypes (G/G 0.24±0.03 vs. A/G 0.33±0.02 and A/A 0.36±0.02; P<0.05) and this polymorphism influenced insulin- stimulated glucose disposal (G/G 264±28, A/G 233±14 vs. A/A 294±13; P<0.05), nonoxidative glucose metabolism (G/G 146±27, A/G 128±13 vs. A/A 187±13; P<0.05) in muscle and VO_2max (G/G 24±2, A/G 24±1 vs. A/A 29±1; P<0.05). Finally, this DNA methylation site was only methylated in elderly and in none of the young twins carrying the *G/G* genotype and the degree of DNA methylation correlated negatively to muscle NDUFB6 expression ($r=-0.61$; $P<0.05$).

Conclusion: Our data show that OXPHOS expression in human muscle is influenced by genetic, epigenetic and non-genetic factors (e.g. age), thereby providing an example how genes and environment may interact to increase age-dependent susceptibility to type 2 diabetes.

Supported by Swegene

OP 34

Molecular mechanisms of obesity

0199

Obese are resistant to the catabolic, but not to the cognitive effects of central nervous insulin

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Background and Aims: Insulin acts in the central nervous system to reduce food intake and body weight and is considered a major adiposity signal. Intranasal insulin administration increases cerebrospinal fluid concentrations of the peptide without absorption into the blood stream. In a previous study, eight weeks of intranasal insulin administration induced loss of body weight and body fat and improved declarative memory in lean men. The present experiments aimed to generalize these findings to obese subjects.

Materials and Methods: Insulin and placebo, respectively, were intranasally administered 4 times a day (amounting to 160 IU/d) over a period of 8 weeks to two groups of 15 obese men each in a double-blind manner (body mass index, mean±SEM: insulin: 32.24±1.21; placebo: 33.13±1.50 kg/m^2). Body weight and body composition as well as declarative memory (recall of word lists) were evaluated during baseline and at the end of the treatment period.

Results: Contrasting with the catabolic effects in normal-weight men, insulin treatment did not induce any significant reduction of body weight and body fat in the obese subjects. However, in accordance with the effects in normal-weight men, declarative memory was improved (remembered words, placebo: 1.13±0.38; insulin: 2.87± 0.56, p<0.05) and hypothalamic–pituitary–adrenal axis activity in the morning was reduced in obese subjects (ACTH, placebo: 25.65±3.62; insulin: 20.11±3.27 pg/ml, p<0.01; cortisol, placebo: 13.03±1.42; insulin: 9.55± 0.55 μg/dl, p<0.04).

Conclusion: Our results indicate that intranasal insulin is functionally active in obese men, inducing an improvement in declarative memory, but fails to affect the neuronal networks critically involved in body weight regulation. They confirm for the first time in humans that obesity is associated with diminished central nervous sensitivity to the adiposity signal insulin. This defect likely contributes to the persistence of obesity in spite of elevated levels of circulating insulin in these patients.

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0200

Adiponectin and resistin in human cerebrospinal fluid and disturbance of the blood brain barrier with obesity

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Background and Aims: The adipocytokines adiponectin and resistin are both implicated in the pathogenesis of obesity mediated diabetes but central action in humans is unknown. In contrast, the adipocytokine leptin has been extensively studied in regards of its central appetite regulating pathways, with leptin resistance identified in obesity. The aims of these studies were 1) to examine serum

and CSF levels of adiponectin and resistin and their relationship with age, gender, body mass index (BMI), insulin resistance (IR) and parameters of inflammation 2) to compare their CSF/serum ratio with leptin and 3) to establish the presence of adiponectin receptors (AdipoR1 & R2) in the human hypothalamus.

Materials and Methods: Serum and CSF samples were analysed from 39 consenting non-diabetic Caucasian subjects undergoing elective surgery with spinal anaesthesia. In total, 20 men (Age: 69.8±(SD)8.6 years; BMI: 29.4(SD)± 3.4 kg/m^2) and 19 women: (Age: 69.4±4.3 years, BMI: 27.3±4.8 kg/m^2) were analysed for adiponectin, resistin, leptin, insulin and high sensitivity (hs) CRP levels. Further, human brain slices were analysed for ACRP 1 and 2 by immunohistochemistry. The HOMA index was used as a measure of IR.

Results: The mean total CSF adiponectin level was 16.4±6.25 pg/ml. Serum and CSF adiponectin levels correlated positively with serum levels (r=0.34; p=0.047). Serum and CSF adiponectin levels showed a positive correlation with age (CSF r=0.36, p=0.035). Serum adiponectin levels were significantly higher in females than in males (p=0.015), but CSF levels were not gender. Serum resistin levels correlated with age (r=0.45, p=0.006), but not in CSF levels (mean 0.2±0.13 ng/ml). Serum adiponectin but not resistin levels showed an inverse correlation with HOMA index (serum Adiponectin r=−0.36, p=0.036), whilst no pattern was observed for CSF levels. CSF Adiponectin and resistin appeared unrelated to hsCRP.

In males, serum and CSF adiponectin showed a significant, negative correlation with BMI, which remained significant even when age was adjusted for (serum: r=−0.569, p=0.01, n=17; CSF: r=−0.62, p=0.01, n=14). The CSF/Serum ratio was inversely correlated with BMI in women (r=−0.61, p=0.02) whilst a similar correlation was found for leptin (r=−0.7, p<0.01). Furthermore, adiponectin receptors were identified in the human hypothalamus.

Conclusion: The study demonstrated that both adiponectin and resistin are detectable in human CSF and CSF adiponectin levels are age and BMI dependant. Both adipokines appear to enter CSF by a mechanism yet to be determined -either transported into the CSF from serum or produced within brain. The presence of adiponectin in the CSF suggests a potential regulatory role in energy homeostasis, which may influence body fat mass in humans. As with leptin, there is a gender dependent decrease in the adiponectin serum/CSF ratio with BMI. This suggests alteration of transport into the CSF for adiponectin in obesity is unrelated to insulin resistance and inflammation. These findings implicate a role for these adipokines in energy homeostasis and the cause of changes in obesity.

0201

Lifestyle intervention downregulates the expression of genes linked to extracellular matrix (ECM) and cell cycle in adipose tissue in subjects with metabolic syndrome – the GENOBIN study

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Background and Aims: Lifestyle modification has been proven to be effective in the prevention of type 2 diabetes, although the underlying molecular mechanisms are not well known. The aim of the present study was to examine the effect of a lifestyle intervention on gene expression in adipose tissue and to find out genes and gene clusters contributing to the development of type 2 diabetes.

Materials and Methods: Seventy-five overweight to obese subjects (BMI 28-40 kg/m^2) with impaired glucose tolerance, aged 60±7 years (mean±SD) were randomized either to weight reduction (WR) (N=28), aerobic training (N=15), resistance training (N=14) or control (N=18) groups according to gender, age and BMI. Oral and intravenous glucose tolerance tests, fasting blood samples, anthropometric measurements, and subcutaneous adipose tissue (AT) biopsies were performed before and after the 33-week intervention. Gene expression using microchip technology was studied in AT samples in 9 subjects from the WR and 10 from control group that was confirmed by real time PCR in the whole population. The study was approved by the Ethics Committee of the Kuopio University Hospital and was performed in accordance with the standards of the Helsinki Declaration.

Results: The subjects in WR group decreased body weight (p<0.0001), BMI (p<0.0001), waist circumference (p<0.0001) and lean body mass (p<0.005). No significant changes were seen in other groups. The WR group was divided according to success in weight reduction: ≥5% (Group A, N=11) and <5% (Group B, N=17) of their initial weight. Fasting serum insulin (p=0.027) and fasting plasma glucose (p=0.057) concentrations decreased during the intervention in Group A. The insulin sensitivity index increased more in Group A than in Group B (p=0.037). Downregulation in AT gene expression was evident in Group A (of 105 genes with changes in gene expression 82% were downregulated, p<0.01), whereas an opposite effect was seen in the control group (of 62 genes with

changes in gene expression 82% were upregulated, p<0.01). The changes in gene expression were modest, with fold changes ranging from 0.67 to 1.68 in Group A and from 0.63 to 1.33 in the control group. Gene Ontology (GO) clusters for biological processes were assessed for enrichment of genes in each cluster with p<0.001. Altogether seven GO biological process clusters were found in 105 genes linked to ECM, cell cycle and proliferation in Group A.

Conclusion: Long-term weight reduction resulted in significant overall downregulation in gene expression in AT, especially in genes linked to ECM and cell cycle. This suggests that ECM might have a role in the development of obesity and insulin resistance. Downregulation of genes linked to the cell cycle suggests adaptation also at the molecular level after intensive weight loss.

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0202

Obesity, exercise and variants in the adioponutrin gene: a population based study

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Background and Aims: We have recently shown that obese subjects have an increased adipose adiponutrin (*ADPN*) mRNA expression and that variants in the gene show association with obesity. Carriers of the associated alleles had a lesser increase in adipose mRNA expression but also an increased basal lipolysis. Functional studies show that ADPN has both lipase and transacylase activity *i.e.* working both in a lipogenic and a lipolytic way. Additionally, the *ADPN* mRNA expression is widely regulated by nutritional status. A high carbohydrates diet increases the mRNA expression in adipose tissue. There seem to be an insulin dependent effect on the increased expression seen by glucose in human adipocytes suggesting insulin as an important regulator. The aim of the study was to further investigate the association between *ADPN* and obesity and the possible influence of exercise.

Materials and Methods: The coding SNP rs738409 and the intronic SNP rs2072907 in the *ADPN* gene were genotyped using TaqMan assays on the ABI 7900HT (Applied Biosystems) in a population-based material from Vara, Sweden (n[m/f]=904/905, age 46[58–56] years, BMI 26.0[23.7–29.3] kg/m^2). Information about physical activity and anthropometrical measures were available.

Results: Both SNPs differed significantly in allele frequency where the common allele was over-represented in the obese subjects ($P_{rs738409}$=0.012, $P_{rs2072907}$=0.046). There was significantly more carriers of the homozygous rs738409 common allele among the obese compared to non-obese [*P*=0.030]. Homozygous carriers of rs738409 common allele had an increased risk of obesity, OR 2.26 (1.11–4.59) [*P*=0.024]. The effect was stronger in subjects above 50 years of age, OR 4.82(1.12–20.69) [*P*=0.035]. Homozygous carriers of the common allele had an increased risk of developing obesity when the level of physical activity was low, OR 2.16(1.00–4.67) [*P*=0.049]. There was no significant increased risk of developing obesity when carrying the risk allele when the level of physical activity was high [*P*=0.22]. There was no significant effect of rs2072907 although the trends were similar to those of rs738409. Grouping carriers of the rs2072907 variant allele resulted in a borderline significant higher risk of obesity if you are a homozygous carrier of the common allele, OR 1.28(0.99–1.65) [*P*=0.056].

Conclusion: This study confirms the previous association between the *ADPN* SNPs and obesity. Homozygous carriers of the common allele have an increased risk of obesity but an increased physical activity might lower this risk. It still remains to be clarified if this is an actual effect of the workout itself or if it is due to concomitant dietary habits. The functionality of the coding SNP rs738409 may be of importance for future studies.

0203

Single nucleotide polymorphisms in the Krüppel-like factor 7 (*KLF7*) gene are involved in the pathogenesis of obesity

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Background and Aims: The *KLF7* gene encodes Krüppel-like factor (KLF) 7, which is a member of the KLF family of transcription factors, initially shown to play important roles in cellular development and differentiation, and recently reported to be involved in adipogenesis. Several single nucleotide polymorphisms (SNPs) have been identified in the *KLF7* gene, of which some have been associated

with type 2 diabetes in a Japanese population; however, a possible association of *KLF7* SNPs with obesity has not been investigated. The aim of this study was to examine *KLF7* SNPs in relation to obesity and associated metabolic quantitative traits in Danish whites. Two intronic SNPs (rs1263608 and rs1263627) were initially investigated, whilst studies of interactions with other SNPs in the *KLF7* gene are ongoing.

Materials and Methods: Studies were performed in the population-based Inter99 cohort of middle-aged people (n=5,570; 2,803 men, 2,767 women). 1,182 of these subjects (21%) were obese (BMI≥30 kg/m^2). The two SNPs were genotyped using the PCR-Invader assay.

Results: The *KLF7* variants rs1263608 and rs1263627 were associated with obesity in case-control studies. The minor allele frequency of rs1263608 was higher among obese subjects (BMI ≥ above 30 kg/m^2) than among control subjects (BMI <30 kg/m^2) (39.8% [95% CI: 37.6–42.0%] vs. 37.2% [36.2–38.1%], p=0.03, odds ratio 1.17 [1.01–1.36] when carrying the minor allele); this was also the case for rs1263627 (28.8% [26.8–30.9%] vs. 25.9% [25.0–26.8%], p=0.009, odds ratio 1.22 [1.06–1.41] when carrying the minor allele). Further evidence of an association with obesity was found in an analysis of quantitative traits, where subjects carrying the minor alleles of either rs1263608 or rs1263627 had a higher BMI than wild type subjects (p=0.006 and p=0.003 for the two SNPs, respectively). Finally, all carriers of the rs1263608 variant had statistically significant elevated levels of serum insulin 30 min post-OGTT (p=0.03), and the incremental area under the curve for circulating levels of glucose, insulin, and C-peptide during a 2-h OGTT was also elevated in these subjects (p=0.04, 0.02, and 0.04, respectively).

Conclusion: In a large study sample of Danish whites we identified an association between SNP rs1263608 and rs1263627 in *KLF7* and obesity. Studies of interactions with other SNPs in the gene are ongoing.

0204

Variants in the hepatic lipase gene (*LIPC*) associate with obesity and elevated fasting plsama HDL-cholesterol levels in a population based sample of 5,850 middle-aged people

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Background and Aims: High density lipoprotein (HDL) is involved in the transport of cholesterol from peripheral tissues to the liver, where it is metabolised. Hepatic lipase (HL) is a liver-specific lipolytic enzyme, catalyzing the hydrolysis of triglycerides and phospholipids in HDL particles. Promoter variants of the gene encoding HL (*LIPC*) have been associated with lower HL activity. Thus, the aim of the study was to elucidate if variants in *LIPC* associate with obesity and related quantitative metabolic traits. We also hypothesized that lifestyle factors modify the effect of genotype.

Materials and Methods: We genotyped the *LIPC* -250G>A promoter (rs2070895) and the Ser215Asn (rs6083) polymorphisms in 5,850 individuals from a population-based cohort (Inter99) using mass-spectrometry of PCR products and TaqMan allelic discrimination.

Results: We observed no linkage disequilibrium between the two variants (D′=0.05; r^2=0.001). The Ser215Asn variant associated with obesity (BMI≥30 kg/m^2) in a case-control study of 2,585 lean and 966 obese individuals (minor allele frequency 36.7% [95% CI: 35.4–38.0%] vs. 39.7% [37.5–41.9%]; odds ratio 1.14 [1.02–1.27]; p=0.019), and with elevated BMI in a study of quantitative traits (additive model: p=0.016). The −250G>A polymorphism showed no association with obesity, yet carriers of the A allele (A allele frequency 22.6% [95% CI: 21.8–23.4%]) had increased fasting plasma HDL-cholesterol levels compared to subjects with the GG genotype (GG: 1.42±0.39 mmol/L, GA: 1.46±0.41 mmol/L, AA: 1.53±0.44 mmol/L; additive model: p=7 *10^{-10}). Moreover, we observed a significant interaction of smoking habits with the −250G>A variant, indicating a greater effect of smoking on lowering the HDL-cholesterol levels in subjects with the GG or GA genotype compared to subjects with the AA genotype (GG/GA: −0.11 mmol/L vs. AA: 0.01 mmol/L; recessive model: p=0.01).

Conclusion: The *LIPC* 215Asn allele associates with obesity and the −250G>A polymorphism associates with plasma HDL-cholesterol levels influenced by interaction of smoking status. This study underlines that variation in *LIPC* modulates risk factors of cardiovascular disease.

OP 35

Second messengers and oscillatory behaviour in beta cell function

0205

Glucokinase and PFK-2/FBPase-2 interact to fulfil crucial roles in glucose induced insulin secretion in pancreatic beta cells and in glucose disposal in liver

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Background and Aims: Glucokinase (GK) plays a pivotal role in the process of glucose recognition in pancreatic beta cells and in glucose metabolism in liver. The bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK-2/FBPase-2) is a key regulator in liver metabolism. Pancreatic beta cells express the PFK-2/FBPase-2 brain isoform. In previous experiments we elucidated the binding between both proteins. Interestingly, PFK-2/FBPase-2 activates GK *in vivo* irrespective of the isoform. Therefore, it was the aim of this study to elucidate the underlying molecular mechanisms of the GK-PFK-2/FBPase-2 complex formation.
Materials and Methods: Interaction between recombinant GK protein and recombinant PFK-2/FBPase-2 or FBPase-2 protein was analyzed with *in vitro* pull-down assays, fluorescence spectroscopy and steady state kinetics. Intracellular binding of the enzymes were analyzed in GK and PFK-2/FBPase-2 overexpressing RINm5F cells by immunohistochemistry.
Results: Incubation with recombinant liver PFK-2/FBPase-2 for 1 h *in vitro* resulted in a significant 3 fold increase in GK enzyme activity. In a Ni-NTA affinity assay recombinant His-tag GK was able to pull-down AlexaFluor-594 labeled FBPase-2 and addition of unlabeled FBPase-2 confirmed that the binding is specific and reversible. In vitro titration of AlexaFluor-594 labeled PFK-2/FBPase-2 with GK labeled by AlexaFluor-488, while following the FRET signal between the fluorescence dyes, suggests a 1:1 stoichiometric GK-PFK-2/FBPase-2 complex. In insulin-producing RINm5F cells GK and PFK-2/FBPase-2 immunofluorescence was detectable in the cytoplasm. Quantitative analyses revealed a significant 3 fold increase of colocalization between both proteins in cells cultured at 10 mmol/l glucose in comparison to cells cultured at 3 mmol/l glucose.
Conclusion: Glucose promotes the GK-PFK-2/FBPase-2 interaction. This indicates a glucose-dependent stabilization of a specific GK conformation by PFK-2/FBPase-2 and a major regulatory principle of the GK-PFK-2/FBPase-2 complex in glucose sensing cell types. In beta cells activation of the glucose sensor GK stimulates glucose-induced insulin secretion. In liver GK stabilization mediates a coordinated upregulation of the glucose disposal. Our results may open the perspective for a therapeutic targeting of this interaction in type 2 diabetes therapy.
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0206

Synchronous oscillations of cAMP and Ca^{2+} in hormone-stimulated insulin-secreting β-cells

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Background and Aims: Ca^{2+} signaling has been extensively characterized and found to involve oscillations important for the temporal control of insulin secretion. In pancreatic β-cells, cAMP affects the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) and amplifies Ca^{2+}-dependent insulin release. The hormones glucagon and glucagon-like peptide-1 (GLP-1), strongly amplify nutrient-stimulated insulin secretion by raising cAMP and modulating Ca^{2+} signaling. We therefore studied the kinetics of hormone-evoked cAMP and Ca^{2+} signaling in insulin-secreting β-cells.
Materials and Methods: We developed a fluorescent biosensor that reports the cytoplasmic cAMP concentration beneath the plasma membrane ($[cAMP]_i$). Insulin-secreting INS-1 cells were transfected with a truncated form of the protein kinase A (PKA) regulatory subunit fused with CFP and targeted to the plasma membrane. Co-expressed YFP-tagged PKA catalytic subunit also localized to the membrane under basal conditions, but dissociated upon elevation of $[cAMP]_i$. This translocation was detected as an increase of the CFP/YFP fluorescence ratio. Simultaneous recording of $[cAMP]_i$ and $[Ca^{2+}]_i$ was performed with evanescent wave microscopy and the fluorescent indicator fura-red.
Results: An increase in $[cAMP]_i$ evoked by IBMX inhibition of phosphodiesterases or forskolin stimulation of adenylyl cyclases resulted in rapid and pronounced rise of the CFP/YFP fluorescence ratio. Stimulation of INS-1 cells with 0.3–10 nM glucagon or GLP-1 evoked pronounced oscillations of $[cAMP]_i$. These $[cAMP]_i$ oscillations disappeared upon removal of extracellular Ca^{2+} and this effect was readily reversible. Simultaneous recording of $[cAMP]_i$ and $[Ca^{2+}]_i$ from GLP-1 stimulated cells revealed that the initial $[cAMP]_i$ elevation was followed by rise of $[Ca^{2+}]_i$, and that subsequent $[cAMP]_i$ oscillations were synchronized with oscillations of $[Ca^{2+}]_i$.
Conclusion: These data demonstrate receptor-triggered oscillations of cAMP synchronized with those of Ca^{2+}. The interplay between cAMP and Ca^{2+} should increase the signal information content improving low-level signal detection and further contribute to the specificity of the downstream cellular responses like ion channel activity, insulin secretion, β-cell survival and proliferation.

0207

The temporal pattern of cAMP signals determines protein kinase A activity range in insulin-secreting cells

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Background and Aims: Stimulation of insulin-secreting cells with glucagon or glucagon-like peptide-1 (GLP-1) has been found to cause oscillations of the cAMP concentration beneath the plasma membrane ([cAMP]). To investigate whether [cAMP] oscillations can contribute to selective regulation of downstream cellular targets we studied the effect of different time-courses of [cAMP] signals on the generation of cytoplasmic Ca^{2+} signals and on the nuclear translocation of the protein kinase A (PKA) catalytic subunit in individual INS-1 β-cells.
Materials and Methods: [cAMP] was measured with a novel fluorescent translocation biosensor and ratiometric evanescent wave microscopy. The cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured with ratiometric epifluo rescence imaging and the fura-2 indicator. Analysis of PKA nuclear translocation was performed with epifluorescence imaging and FlAsH-labelling of tetracysteine-tagged PKA-Cα subunit.
Results: Stimulation of INS-1 cells with GLP-1 induced dose-dependent increases of [cAMP] with oscillations in >80% of the cells at 0.3–1 nM, whereras higher concentrations of the hormone most frequently elicited stable [cAMP] elevations (>60%). Inhibition of phosphodiesterases with 100 μM IBMX resulted in a prompt rise ($t_{1/2}$=23±2 s) of [cAMP], consistent with a high basal rate of cAMP formation. The IBMX-induced elevation of [cAMP] lasted throughout the stimulation period but rapidly returned to basal levels upon removal of the stimulus ($t_{1/2}$=14±3). Pulsatile application of IBMX (1 min stimulation, 3 min wash) generated oscillations of [cAMP] mimicking those observed after hormone stimulation. Both stable and pulsatile application of IBMX induced pronounced $[Ca^{2+}]_i$ spiking and in the case of [cAMP] oscillations the $[Ca^{2+}]_i$ spikes were grouped and correlated with the periods of elevated [cAMP]. In contrast, only prolonged stable [cAMP] elevation induced nuclear entry of FlAsH-labelled PKA-Cα subunit.
Conclusion: These data indicate that cAMP oscillations can contribute to selective regulation of downstream targets by restricting the spatial redistribution of PKA.

0208

Oscillations of PIP3 in insulin-secreting cells evoked by glucose- and insulin-induced co-activation of PI3-kinase

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Background and Aims: Glucose-induced insulin release from pancreatic beta-cells is associated with autocrine activation of insulin receptors on the beta-cell surface, which leads to stimulation of PI3-kinase and formation of the lipid messenger phosphatidylinositol-3,4,5-trisphosphate (PIP3). PIP3 regulates many important cellular functions, including gene transcription, cell survival and proliferation as well as insulin secretion. The present study aimed at investigating the time-course of PIP3 signals evoked by glucose in individual beta-cells.
Materials and Methods: Insulin-secreting MIN6 cells were transfected with the pleckstrin homology domain from Akt fused to GFP (GFP-AktPH). This biosensor resides in the cytoplasm under basal conditions but redistributes to the plasma membrane upon generation of PIP3. The translocation was detected with evanescent wave microscopy and the cytoplasmic Ca2+concentration ([Ca2+]i) was simultaneously measured with the fluorescent Ca2+indicator fura red.
Results: MIN6 cells responded to 100 nM insulin with GFP-AktPH translocation resulting in a 30±3% (n=42) increase of plasma membrane fluorescence. Depolarization with 30 mM KCl caused rapid elevation of [Ca2+]i, which was paralleled by a 28±3% (n=21) increase of membrane GFP-AktPH fluorescence. Removal of extracellular Ca2 +prevented both the depolarization-induced [Ca2+]i increase and the GFP-AktPH translocation, whereas inhibition of insulin secretion with 5 μM epinephrine abolished the KCl-induced GFP-AktPH translocation without affecting the [Ca2 +]i response. Elevation of the glucose concentration from 3 mM to 20 mM resulted in an initial lowering of [Ca2+]i followed within 3 minutes by a rapid rise, that was associated with an increase of GFP-AktPH membrane fluorescence (48±3%, n=58). After 5–10 min, there was regular oscillations of membrane GFP-AktPH fluorescence (0.22±0.015 min−1, n=28). Each oscillation was preceded by a distinct elevation of [Ca2+]i. In the presence of 250 μM of the hyperpolarizing agent diazoxide, glucose did not induce significant GFP-AktPH translocation by its own, but markedly enhanced the response to 100 nM exogenous insulin (48±5% vs. 28±2, P<0.001, n=42).
Conclusion: It is concluded that glucose and insulin synergistically activate PI3-kinase in insulin-secreting cells and that oscillations of PIP3 occur as a result of pulsatile insulin release.

0209

Plasma membrane PIP_2 concentration is dynamically regulated by Ca^{2+} and the ATP/ADP ratio in insulin secreting cells

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Background and Aims: Phosphatidylinositol 4,5-bisphosphate (PIP_2) is important for a variety of cellular processes by serving as precursor for second messengers and by regulating ion channels, the cytoskeleton, and vesicle traffic in many types of cells, including insulin-secreting β-cells. The present study aimed at investigating how the plasma membrane concentration of PIP_2 is regulated by glucose, intracellular Ca^{2+}, ATP and by different phosphoinositide kinases in individual insulin-secreting cells.
Materials and Methods: Plasma membrane PIP2 concentration was recorded in insulin-secreting MIN6-cells with evanescent wave microscopy and the PIP_2-binding GFP-tagged PH-domain from PLCδ. In some experiments, the cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) was simultaneously measured using the fluorescent indicator fura red.
Results: Elevation of the glucose concentration from 3 to 11 mM evoked anti-synchronous oscillations of [PIP_2] and cytoplasmic Ca^{2+}concentration, consistent with phospholipase C being periodically activated by the voltage-dependent Ca^{2+}-influx. In α-toxin-permeabilized cells, 0.1–1 mM ATP induced dose-dependent increases of [PIP_2] counteracted by the ADP-analogue adenosine-5′-O-2-thiodiphosphate. There was rapid turnover of PIP_2 in the membrane with $t_{1/2}$16 s. RT-PCR analysis identified transcripts of 10 different phosphoinositide-kinase isoforms. Adenosine-sensitive type-II- and wortmannin-sensitive type-III PI4-kinases were equally important for ATP-dependent PIP_2-formation. Moreover, PIP_2 synthesis was selectively enhanced by overexpression of PIP5-kinase Iβ, but not by the α- or γ-isoforms.
Conclusion: We conclude that plasma membrane PIP_2 is dynamically regulated by intracellular Ca^{2+} and the ATP/ADP ratio in insulin-secreting cells. The rapid turnover allows generation of second messengers with maintenance of the PIP_2 levels required for sustained exocytosis of insulin secretory granules.

0210

Unmasking glucose-induced insulin secretion in mouse islets lacking K_{ATP} channels

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Background and Aims: A current model ascribes glucose stimulation of insulin secretion (GSIS) to the cooperation of a K_{ATP} channel-dependent triggering pathway and a K_{ATP} channel-independent amplifying pathway. Closure of K_{ATP} channels leads to β-cell depolarization, increased Ca^{2+} influx and rise of cytosolic Ca^{2+} ($[Ca^{2+}]_c$) that triggers secretion. The amplifying pathway does not produce a further rise of $[Ca^{2+}]_c$, but is thought to augment Ca^{2+} action via still unidentified mechanisms. However, several groups using islets from sulfonylurea receptor 1 knockout (Sur1KO) mice failed to measure significant GSIS in these islets whose β-cells lack K_{ATP} channels. Our aim was to address this issue that casts doubt on the validity of the model of β-cell function.
Materials and Methods: Islets were isolated from adult control and Sur1KO mice (provided by J. Bryan, Houston), and tested rapidly after isolation (2 h preincubation in Krebs medium with 10 mmol/l glucose - G10) or after overnight culture in RPMI medium with G10. The islets were perifused for measurement of IS or $[Ca^{2+}]_c$ or incubated with U-^{14}C-glucose for measurement of glucose oxidation.
Results: Compared with control islets, freshly isolated Sur1KO islets had elevated basal (G1) $[Ca^{2+}]_c$ and IS rate that were both corrected by preventing Ca^{2+} influx. Despite the lack of K_{ATP} channels, they responded to G15 by a transient drop in $[Ca^{2+}]_c$ followed by an increase which, however, remained lower than glucose-stimulated $[Ca^{2+}]_c$ in control islets. The absolute rate of GSIS was ~3-fold lower in Sur1KO than control islets, with only a poor increase above baseline (G15: G1=1.2-fold in Sur1KO vs>20-fold in controls). These differences could not be explained by differences in insulin content, alterations of glucose oxidation or poorer synchronization of $[Ca^{2+}]_c$ oscillations in islets. Overnight culture of Sur1KO islets slightly augmented basal IS (G1) and considerably improved their secretory response. Stimulation with G15 now induced a 4-fold increase in IS above baseline, reaching higher absolute values than in cultured control islets. Importantly, overnight culture did not produce similar effects in control islets, whose GSIS was barely different from that of fresh islets. This major impact of culture on GSIS in Sur1KO islets did not require serum but was markedly attenuated when the RPMI medium contained only G5 instead of G10. It was not attributable to changes in the rate of glucose oxidation, but could at least partly be explained by higher $[Ca^{2+}]_c$ levels in the islets. Testing the amplifying pathway during KCl-depolarization of fresh and cultured Sur1KO islets did not evidence significant differences, indicating that this pathway is functional immediately after isolation provided $[Ca^{2+}]_c$ is sufficiently increased.
Conclusion: Overnight culture of Sur1KO islets unmasks the ability of glucose to increase IS in β-cells lacking K_{ATP} channels. The poor efficacy of glucose in fresh Sur1KO islets (probably reflecting the *in vivo* situation) is attributed

to a weak triggering signal ($[Ca^{2+}]_c$ is low compared to G-stimulated control islets) and a poor efficacy of the amplifying signals (because their action requires higher $[Ca^{2+}]_c$). Altogether, these data help explaining controversies in the literature and support models implicating two pathways in the control of GSIS.

OP 36

New therapies – pre-clinical

0211

Comparative effects of CS-917, a fructose 1,6-bisphosphatase inhibitor, and metformin

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Background and Aims: CS-917 (MB06322) is a prodrug of an inhibitor of fructose 1,6-bisphosphatase, a rate-limiting enzyme of gluconeogenesis (GNG). CS-917 decreases plasma glucose in cynomolgus monkeys and various rodent models of type 2 diabetes (T2DM) by inhibition of GNG. The mechanism of a biguanide, metformin (Met), has not been elucidated in detail although a predominant effect in patients with T2DM is reported to be inhibition of hepatic glucose production through reduced GNG. In the present study, we compared the glucose-lowering mechanisms and effects between CS-917 and Met.
Materials and Methods: 1) Blood glucose kinetics was analyzed using the Steele equation. CS-917 (7.5 mg/kg) or Met (300 mg/kg) was administered orally to overnight-fasted Goto–Kakizaki (GK) rats (insulin deficient model), followed by intravenous injection of ^{3}H-glucose.
2) Glucose-lowering effects in fasted and non-fasted GK rats were evaluated after oral administration of CS-917 (15 mg/kg) or Met (300 mg/kg). The AUC of plasma glucose for 8 h was calculated.
3) *In vivo* efficacy was then evaluated by AM/PM combination treatment regimens in Zucker diabetic fatty rats (insulin resistant model). AM and PM doses were followed by 8 h-daylong fasting and nocturnal *ad lib* feeding, respectively. CS-917 (150 mg/kg) and Met (150 mg/kg) were orally administered AM or PM in one of the AM/PM combination treatment regimens. Glycohemoglobin levels were measured after 6-week treatment.
Results: 1) CS-917 decreased the appearance of blood glucose, but not the disappearance of blood glucose. In contrast, Met increased the appearance and disappearance of blood glucose (see Table 1).
2) CS-917 significantly decreased the AUC of plasma glucose in the fasted state and Met decreased that in the fed state (see Table 1).
3) The regimen of dosing CS-917 AM with Met PM decreased glycohemoglobin levels more than its reverse regimen (AM/PM regimen, glycohemoglobin level (%, mean-SE): control, 9.6±0.4; CS-917/Met, 7.5±0.5*†; Met/CS-917, 8.4±0.4* (*P<0.05 vs. control, $^{\dagger}P$<0.05 vs. Met/CS-917)).

Table 1 Glucose kinetics and effect on AUC of plasma glucose levels in GK rats

Treatment	Glucose rate (mg/dL/min)			AUC of plasma glucose (mg/dLxh)		
	N	Appearance	Disappearance	N	Fasted state	Fed state
Control	11	1.69±0.47	1.26±0.87	6	1758.5±162.3	2472.6±149.4
CS-917	11	0.43±0.16*	1.15±0.30	6	1060.7±13.2*	2284.8±89.0
Met	12	2.85±0.18*	3.45±0.28*	6	1593.0±61.0	2015.6±98.5*

Mean±SE; *P<0.05 vs. control

Conclusion: CS-917 decreased glucose appearance consistent with GNG inhibition. In contrast, Met increased glucose disappearance. The glucose-lowering effect of CS-917 was observed in the fasted state where GNG is predominant. In contrast, that of Met was observed in the feeding state. The repeated bid regimen of CS-917 before fasting with Met before feeding represented better reduction in glycohemoglobin than the reverse dosing regimen. These results suggested that CS-917 and Met have different glucose-lowering mechanisms and that their efficacies are partly dependent on the timing of administration and/or on the feeding state. CS-917 is the first in a new class of oral antidiabetic agents and provides a new therapeutic strategy for the treatment of T2DM.

0212

Evaluation of SVT002149, a novel PPARγ modulator with *in vivo* antiadipogenic properties

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Background and Aims: PPARγ agonists confer anti-diabetic effects in animals and humans. Nevertheless, they are associated with significant adverse effects, including weight gain, edema, mild anemia, risk for congestive heart failure and tumour formation, which limits their clinical use. An alternative approach is the recent discovery of ligands with

partial agonist activity, which offer the promise of retained efficacy and potentially reduced toxicity. The aim of this work is to describe the *in vitro* profile of SVT002149, a PPARγ modulator and its effects in a rodent model of obesity.
Material and Methods: – Binding assay*:* [3H]-rosiglitazone (RSG) and [3H]GW-2433 were used as radioligands for GST-PPARγ, and GST-PPARα/PPARδ, respectively. Bound radioligand was separated by affinity chromatography.
– *Trans-activation assay:* COS-7 cells were transfected with pFACMV-hPPARγLBD/GAL4, pUAS(5×)-tk-luciferase and pRLrenilla. After 48 h exposure to compounds, luciferase activity was determined.
– *3T3-L1 preadipocytes differentiation:* After reaching the confluence, cells were treated with RSG and insulin for 9 days, and stained with *Oil Red O*.
– *Molecular Docking:* Ligands were docked by means of Induced Fit Docking (Schrödinger LLC).
– *Diet Induced–Obese (DIO) Mice:* Male C57BL/6J mice were used. LF control was fed a low fat diet (5% kcal) and the HF control a high-fat diet (45% kcal). Animals were treated with the compound incorporated into the diet. Body weight and food intake were routinely determined for 10 weeks. Plasma and hematology parameters were tested and the necropsy evaluated. The study was performed in accordance with the institutional ethical guidelines.
Results: SVT002149, a compound structurally distinct from glitazones, is a selective PPARγ ligand (Ki=470 nM, compared to RSG, Ki=230 nM). This ligand shows partial agonism in cell-based trans-activation of PPARγ. Even more, it inhibits the trans-activation induced by RSG in a concentration-dependent manner. In comparison with RSG, SVT002149 has a reduced adipogenic activity in 3T3-L1 cells. Induced Fit Docking studies reveal that both molecules interact with PPARγ throught a distinct binding mode: whereas RSG is packed between α-helix 4 and β-sheet 1, SVT002149 binds to a hydrophobic pocket formed by α-helixes 4, 7 and 8, and also to the pocket formed by α helixes 4, 6, β-sheet 1, and loop between α-helixes 2 and 3. These biochemical properties may provide a mechanistic basis for its distinct pharmacological profile: oral treatment of DIO mice with SVT002149 (1–10 mg/kg) is well tolerated, showing an opposite profile to that of RSG: SVT002149 markedly dose-dependent reduced body weight (72%), adiposity index (82%) and hyperinsulinemia (87%). Although neither tryglyceride nor cholesterol accumulation is observed in liver, plasma lipidic profile is significantly improved. Heart weight, plasma biochemical and hematological markers were not affected.
Conclusions: Together, the *in vitro* and the *in vivo* data support that SVT002149 appears to modulate PPARγ response, showing an improvement in insulin- and lipid-lowering efficacy, without eliciting the major side-effects characteristic of full agonists. These results reinforce the concept that selective modulators are attractive drug candidates that may offer an improved therapeutic profile over existing PPAR agonists.

0213

Supplementation with polyphenolic complex from red grapes rehabilitates lipid profile and ameliorates insulin resistance in Golden Syrian hamsters with experimental metabolic syndrome

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Background and Aims: Polyphenolic extract from "Cabernet" grapes (named Enoant, contained approx. 20 g polyphenols per l) has numerous activities, such as antimutagenic, immunopotentiating, cholesterol-lowering, hypotensive and antioxidant effects. We and others have reported that Golden Syrian hamsters can be induced to develop atherosclerosis and metabolic syndrome in a manner that closely mimics human pathology. The aim of the present study was to characterize therapeutic (hypolipidaemic) effect of Enoant in animal model of metabolic syndrome.
Materials and Methods: 30 female 1-mo-old Golden Syrian hamsters were randomized into 3 groups (10 animals each). A control group was submitted to a standard diet. Two groups were submitted for 9 weeks to a diet containing 29% vegetable- and animal oils and fructose (2 g/100 g body weight/day) — metabolic syndrome groups (MS). After than MS groups received Enoant (0.05 ml/100 g body weight, per os, MS+E group) or placebo for the last 14 days. Fasting blood samples were used for glycaemia, plasma insulin, total lipids (TL) and cholesterol, triglycerides (TG), NEFA, LDL +VLDL and α-tocopherol (α-T) levels determinations. TL, α-T, ascorbic acid levels and glucose-6-phosphate-dehydrogenase (G6PDH) activity were measured in liver by spectrophotometrically. Homeostasis Model Assessment (HOMA) was used to estimate insulin resistance (IR). Statistical analyses were carried out using SPSS and Microsoft Excel. Results are expressed as mean±SEM.
Results: IR, hyperinsulinaemia and dyslipidaemia (hypertriglyceridaemia and atherogenic lipid profile) were developed in hamsters fed a half unsaturated fat and fructose-enriched diet compared to controls. Two weeks' oral administration of Enoant to the MS hamsters resulted in marked reduction of serum insulin (by 54%, $p<0.001$), HOMA–IR (8.03±0.73 vs 19.43±2.39, $p<0.001$), TL (3.54±0.16 vs 4.52±0.17 mg/ml, $p<0.001$), TG (91.2±2.3 vs 108.4±4.9 mg/dl, $p<0.01$) and NEFA (0.95±0.02 vs

1.17±0.06 mmol/l, p<0.01) levels in comparison with MS +placebo. This was associated with suppression of G6PDH activity (4.62±0.07 vs 4.96±0.11 nmol NADPH/ min mg protein in MS, p<0.05) and reduction of TL (111.53±4.08 vs 140.75±9.15 mg/g in MS, p<0.01) in liver. Serum and liver α-T contents were also elevated (by 47 and 20.5%, respectively, p<0.01) in MS+E group.
Conclusion: These results show that the abnormal lipid metabolism associated with insulin resistance might be improved in the Golden Syrian hamsters with experimental metabolic syndrome by the oral administration with polyphenolic extract from red grapes. This suggests the potentiality of the Enoant as a novel agent for the treatment of the metabolic syndrome and lipid abnormalities.

0214

Long-term treatment with GLP-1 analogue liraglutide reduces body weight and improves glucose tolerance in diet-induced obese rats more efficaciously than conventional doses of sibutramine and rimonabant

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Background and Aims: The GLP-1 analog liraglutide is currently in late stage clinical development for a once daily treatment of people with type 2 diabetes (T2DM). Beyond improvement of glycaemic control, chronic administration of GLP-1 agonists confer reduction of body weight.
Materials and Methods: We have compared weight reducing efficacy of liraglutide (200 and 300 microg/kg BID) with conventional doses of two oral weight control agents rimonabant (10 mg/kg QD) or sibutramine (5 mg/kg). Adult male diet-induced obese Sprague Dawley (DIO) rats with impaired glucose tolerance were dosed for 28 days.
Results: Liraglutide treatment significantly reduced cumulated food intake (both after 14 and 28 days) and significantly more so than sibutramine and rimonabant. Resulting loss in body weight was twice as large for liraglutide compared to sibutramine and rimonabant. Liraglutide and sibutramine significantly decreased all examined body fat depots (mesenterial, perirenal, epididymal, inguinal), whilst rimonabant only decreased inguinal and epididymal depots. Compared to vehicle, fasting plasma glucose levels and HbA1c levels were unaffected in all treatment groups at the final day of treatment. Plasma triglyceride levels decreased in all treatment groups. On the final day of dosing, an oral glucose tolerance test was performed. Liraglutide was the only treatment which significantly improved glucose tolerance in DIO rats, whereas rimonabant treated animals displayed impaired glucose tolerance. However, all treatments significantly lowered oral glucose induced insulin secretion with sibutramine being less efficacious than liraglutide and rimonabant.
Conclusion: Based on these data it is concluded that liraglutide is a promising weight loss agent having more potent effect on body weight than both rimonabant and sibutramine.

Liraglutide treatment reduces adiposity

Day 28 data	Body weight gain (%)	s.c. inguinal (g)	Epididymal (g)	Mesenterial (g)	Perirenal (g)
Vehicle	105.3±0.9	11.4±0.6	12.8±0.8	12.5±0.9	25.1±1.0
Liraglutide	90.4±0.8 [abc]	7.0±0.6 [ac]	9.4±0.8 [a]	6.8±0.4 [a]	14.9±1.2 [abc]
Sibutramine	96.6±1.0 [a]	8.2±0.5 [a]	10.6±0.7 [a]	8.5±0.7 [a]	18.9±1.2 [a]
Rimonabant	96.1±0.7 [a]	9.7±1.0	10.6±0.7 [a]	8.64±0.8 [a]	18.1±1.3 [a]

[a] p<0.05 vs. vehicle, [b] p<0.05 vs. sibutramine, [c] p<0.05 vs. rimonabant

0215

Abnormal feeding behavior and ghrelin secretion in mice lacking glial cell line-derived factor family receptor GFRα2

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Background and Aims: Neurturin signaling via GDNF-family receptor GFRα2 is required for development and target innervation for subsets of parasympathetic and enteric neurons. Knockout of the receptor gene (Gfra2−/−) (ko) in mice leads to postnatal growth impairment by an unknown mechanism. Ko mice have deficient innervations, impaired function of exocrine and endocrine pancreas and postnatal growth retardation. In ko mice, the growth deficit (~50% compared to wild type mice littermates (wt)) is most pronounced shortly after weaning, but they regain growth during the following months, yet remain app. ~20% smaller than their wt littermates.
Materials and Methods: In this study, we analyzed in detail the feeding behavior and energy expenditure using an automated monitoring system with indirect open circuit calorimeter, food and water consumption recorded in parallel with metabolic measurements in intervals of 15 min and activity measurement by an array of infrared beams. Ghrelin and leptin plasma levels were analyzed by commercially available RIAs.

Results: Total food intake and locomotor activity did not differ between genotypes. However, in ko mice, the duration of individual meals was ~50% longer, meal sizes were reduced by ~40%, and the number of feeding bouts during dark period was increased by ~40% compared to wt mice. In addition, the average time between individual meals during the night phase was significantly shorter in GFRα2-ko mice (ko 12 min vs. wt 20 min) suggesting decreased satiety. The biochemical analysis demonstrated equal glycogen levels in the livers. Determination of peptide hormones involved in food intake regulation we found significantly increased plasma ghrelin levels in both fasted (~+35%) and in fed (~+51%) ko mice compared to their wt control littermates. Feeding reduced ghrelin levels by 45% in the ko mice and 50% in the wt littermates. Plasma leptin levels were reduced by 40% (from 10,2 in wt to 6,1 ng/ml in ko mice) in the fed state.

Conclusion: Our data suggest that elevated ghrelin levels promotes the increase in meal frequency, possibly compensating for the lack of food induced satiety in adult GFRα2-ko mice. Previously, it has been shown that ghrelin secretion is mostly of gastric origin and under cholinergic control. Since our ko mice have deficient peripheral cholinergic innervations, this control seems to be overridden in our mice suggesting that high ghrelin levels are a superior physiological adaptation mechanism in order to increase food intake as indicated by the increased feeding bouts. Analysis of young animals is ongoing to study the possible development of compensatory modifications in feeding behaviour and energy expenditure.

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0216

Leptin mediates sodium tungstate antiobesity effects

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Background and Aims: Oral administration of sodium tungstate in diet-induced obese rats reduces body weight gain and adiposity, without altering food intake. Moreover, it improves the lipid profile and insulin resistance. These effects are due, at least partially, to an increase in energy expenditure and in lipid oxidation. A reduction in blood leptin levels was observed in treated obese animals. Since leptin is a key regulator of energy homeostasis, this finding suggests that leptin could play a major role in the tungstate effect. The aim of this study is to confirm the implication of leptin on the tungstate antiobesity effects.

Materials and Methods: 12 weeks old leptin deficient ob/ob mice and their respective controls (ob/+) were divided in two groups: one was treated with tungstate (2 g/l in the drinking water) for 30 days (*T*), and the other was left untreated (*UT*). The body weight (*BW*) and food intake (*FI*) were measured daily. The oxygen consumption was determined by indirect calorimetry in untreated animals after one tungstate dose (60 mg/Kg) administered by gavage. 500 mg epididimal adipose tissue (*epWAT*) from ob/+mice were divided in four explants and transplanted into ob/ob mice scapular and lumbar subcutaneous zone. The animals were divided in two groups: untreated (*txUT*) and tungstate treated (2 g/l for four weeks, *txT*). An additional group of ob/ob mice transplanted with ob/ob adipose tissue was used as a leptin deficiency control (*txCN*). At the end of the treatment, fat transplants were removed and the treatment was carried out for an additional two weeks.

Results: Tungstate treatment significantly reduces BW gain *(3.23±0.33 vs. 0.7±0.86 g BW gain UT vs. T; p-value <0.05)*, adiposity *(643±78 vs. 416±56 mg epWAT UT vs. T; p-value <0.05)* and FI *(105.2±6.6 vs. 87.9±6.7 g chow/30 days UT vs. T; p-value <0.05)*, and increases oxygen consumption in ob/+mice *(100.0±6.6 vs. 116.8±3.4%O_2 consumption UT vs. T; p-value <0.05)*. However, none of these parameters are significantly changed in treated ob/ob mice *(6.67±0.46 vs. 6.11±0.56 g BW gain, 3430±180 vs. 3450±150 mg epWAT, 149±19 vs. 134±13 g chow/30 days and 100.0±8.0 vs. 87.2±2.9%O_2 consumption UT vs. T; p-value NS in all measurements)*. Ob/ob mice transplanted with epWAT from ob/+have detectable blood leptin levels and show a decrease in body weight gain, compared to transplanted control mice *(−2.32±010 vs. 4.23±0.02 g BW gain txUT vs. txCN; p-value <0.05)*. In addition, food intake is also reduced in these animals *(137.6±9.8 vs. 158.8±9.8 g chow/4 weeks txUT vs. txT; p-value <0.05)*, and oxygen consumption is increased *(1153±24 vs. 1072±20 ml O_2/h/Kg$^{0.75}$ txUT vs. txT; p-value <0.05)*. These effects are amplified in tungstate treated transplanted mice *(−8.10±0.05 g BW gain, 113.9±5.8 g chow/4 weeks and 1280±15.3 ml O_2/h/Kg$^{0.75}$ txT; p-value <0.05 vs. txUT),* indicating that leptin presence is necessary for tungstate to have antiobesity effects. Moreover, morphological differences in brown adipose tissue were observed in transplanted untreated ob/ob mice, which are more accentuated in treated animals. Finally, the removal of transplanted adipose tissue from the treated mice immediately halts tungstate antiobesity effects. These animals show an increase in food intake which leads to raise in body weight.

Conclusions: Tungstate needs leptin presence to increase energy expenditure and to reduce food intake, leading to an antiobesity effect.

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OP 37

Hypertension

0217

Aliskiren, alone and in combination with ramipril, significantly lowers blood pressure and plasma renin activity in patients with diabetes and hypertension, regardless of the degree of glycaemic control

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Background and Aims: Hypertension, a common comorbidity in patients with diabetes, greatly increases the risk of cardiovascular and renal disease. While glycaemic control is the primary objective in diabetes management, morbidity and mortality are also reduced by effective blood pressure (BP) control. We investigated antihypertensive efficacy of the novel renin inhibitor aliskiren and the ACE inhibitor ramipril, alone and in combination, in patients with different degrees of glycaemic control (indicated by glycosylated haemoglobin [HbA1c] levels). Plasma renin activity (PRA; capacity for generation of angiotensin), was measured in a subset of patients from each HbA1c subgroup to determine treatment effects on renin system activation.

Materials and Methods: Patients with type 1 or 2 diabetes and mean sitting diastolic BP (MSDBP) 95−109 mmHg were randomized to double-blind aliskiren 150 mg (n=282), ramipril 5 mg (n=278) or aliskiren/ramipril 150/5 mg (n=277) once daily for 4 weeks, followed by forced titration to aliskiren 300 mg, ramipril 10 mg or aliskiren/ramipril 300/10 mg for a further 4 weeks. Reductions in MSDBP and mean sitting systolic BP (MSSBP) were assessed in patient subgroups with baseline HbA1c < and ≥ median (n=400 and 426, respectively, of whom 121 and 107, respectively, had PRA measured).

Results: Median HbA1c at baseline was 7.0%, which is also the goal for glycaemic control recommended by the American Diabetes Association. Mean HbA1c levels in the two subgroups were 6.2% (range 4.6−6.9%) and 8.2% (7.0−13.8%). Baseline MSSBP and MSDBP were comparable in the two subgroups. Table 1 shows BP reductions at endpoint. Both subgroups showed similar patterns of BP lowering to the overall population; combination therapy was generally superior to ramipril monotherapy. From geometric mean baseline values of 0.45 and 0.51 ng/mL/h in patients with HbA1c < and ≥7%, respectively, aliskiren reduced PRA by 62% and 75%, respectively, as monotherapy, and by 46% and 51% when combined with ramipril. Ramipril monotherapy increased PRA by 92% and 118%, respectively.

Conclusion: Aliskiren monotherapy provides effective BP lowering and renin system suppression. When combined with ramipril, aliskiren provides additional BP lowering and neutralizes ramipril-induced increases in PRA. These effects are independent of the degree of glycaemic control at baseline. Aliskiren may offer the potential of enhanced end-organ protection in patients with hypertension and diabetes, including those with poor glycaemic control at greatest risk of cardiovascular and renal disease.

Least squares mean change from baseline (mmHg)

		Aliskiren	Ramipril	Combination
Overall population	MSSBP	−14.6*	−11.9	−16.5***
n=826	MSDBP	−11.3	−10.7	−12.7†**
HbA1c <7.0%	MSSBP	−14.9	−11.8	−15.6*
n=400	MSDBP	−11.3	−10.8	−13.3†*
HbA1c ≥7.0%	MSSBP	−14.9	−12.1	−17.8***
n=426	MSDBP	−11.5	−10.8	−12.4

*p≤0.05, **p≤0.01, ***p≤0.001 vs ramipril; †p≤0.05 vs aliskiren

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0218

The Anglo–Scandinavian Cardiac Outcomes Trial: Blood Pressure-Lowering Limb (ASCOT–BPLA): effects in patients with type 2 diabetes

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Background and Aims: Diabetes (DM) and hypertension (HT) are both important risk factors for cardio–vascular disease. Despite this very few large trials have been specifically oriented towards patients with DM and HT. The Anglo–Scandinavian Cardiac Outcomes Trial (ASCOT) compared two antihypertensive treatment strategies for the prevention of coronary heart disease (CHD) in more than 19,000 hypertensive patients who had no history of CHD. This report is an analysis of the large subpopulation with DM in this study.
Materials and Methods: Patients were between 40 and 79 years with either untreated HT, systolic blood pressure (SBP) of 160 mmHg or more, and/or diastolic (DBP) of 100 mmHg or more, or treated HT with SBP of 140 mmHg or more, and/or DBP 90 mmHg or more. For those with type II DM at least two additional risk factors were required. Patients were randomised to an amlodipine± perindopril or atenolol ± thiazide regimen with possible addition of doxazosin and other antihypertensives in both arms. Therapy was titrated to achieve a target blood pressure <130/80 mmHg.
Results: The trial was terminated early on recommendation of the safety committee due to benefit in mortality and stroke for the amlodipine based regimen. In the 5137 patients with DM the amlodipine based treatment significantly lowered the incidence of combined major cardio–vascular events including coronary events, strokes and interventional procedures as compared to the atenolol based regimen (hazard ratio 0.86, CI 0.76–0.98), (p=0.026). Fatal and non-fatal stroke was 25% lower (p=0.017), peripheral arterial disease 48% lower (p=0.004) and non-coronary revascularisation procedures 57% lower (p<0.001) in the amlodipine group. For the other endpoints included in the composite endpoint differences were moderate and non-significant. Non-fatal myocardial infarction was reduced by 11% (n s). The absolute risk reduction in major cardio-vascular events by amlodipine±perindopril was very similar in the non-diabetic (2.6%) and diabetic (2.4%) subgroups in ASCOT. Blood pressure (BP) was slightly (1–3 mm Hg) more reduced by treatment based on amlodipine. By the end of the study in those allocated to amlodipine BP was 136/75 mm Hg and in those allocated to atenolol 137/76 mm Hg.
Conclusion: In the diabetic subgroup in ASCOT, treatment with a regimen based on amlodipine±perindopril as compared to atenolol±thiazide reduced the incidence of first total cardiovascular events and procedures by 14%. The finding of this analyses is well in line with the overall finding of a 16% risk reduction of first total cardiovascular events and procedures by treatment based on amlodipine± perindopril in the overall population of ASCOT.
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0219

CYP2C9 polymorphism modifies blood pressure lowering response to losartan in type 1 diabetic nephropathy

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Background and Aims: CYP2C9 of the cytochrome P450 superfamily catalyzes the metabolism of the antihypertensive treatment (AHT), losartan, to its active metabolite E-3174. Two functional polymorphisms within the *CYP2C9* gene result in the expression of three variants, CYP2C9 *1, *2 (Arg144Cys) and *3 (Ile359Leu) encoding enzymes with declining catalytic activity. We investigated whether the CYP2C9 genotype predicts blood pressure (BP) and albuminuria lowering response and preservation of GFR during long-term treatment with losartan.
Materials and Methods: The CYP2C9 alleles were determined in 60 hypertensive type 1 diabetic patients with overt diabetic nephropathy. After a 4 week wash-out period, the patients received losartan (100 mg o.d.) for a mean follow-up of 36 months. Additional AHT was prescribed after 4 months if BP was >135/85 mmHg. At baseline, after 2 and 4 months, and every 6 months thereafter, 24-h BP, albuminuria, and glomerular filtration rate (GFR) were measured. The CYP2C9 genotypes were determined by TaqMan technique.
Results: The CYP2C9*1, *2 and *3 allele frequencies were 78%, 12%, and 10%, respectively. At baseline, 24-h BP, albuminuria, and GFR did not differ between the group of genotypes carrying the *3 allele (n=12, moderate metabolizers) compared to the non-*3 allele carriers (n=48, high metabolizers). After 4 months the non-*3 carriers had obtained a significantly greater reduction in systolic 24-h BP than *3-carriers (mean (SE) mmHg) −15 (16) and 5 (17), respectively (p=0.001) and a tendency to a greater reduction in plasma aldosterone levels (geometric mean (95% CI) %) −24 (−4 to −41) and 22 (−50 to 195) (p=0.13), respectively.
After addition of amlodipine and diuretics in 2 out of 3 patients in both groups, systolic 24-h BP was significantly

lowered in patients carrying the *3 allele, p=0.004, whereas it remained unchanged in non-*3 allele carrying patients. During long-term treatment, albuminuria (p=0.9), or rate of decline in GFR (median (range)) 3.3 (−0.1 to 8.0) ml/min/year and 3.0 (−1.6 to 16.0) (p=0.4) declined similarly in *3 allele carriers and non-*3-carriers, respectively.
Conclusion: The CYP2C9*3 polymorphism is associated with attenuated BP response to losartan treatment. However, by adding other AHT a similar progression of diabetic nephropathy was obtained in Type 1 diabetic patients with or without the CYP2C9*3 allele.

0220

Anti-hypertensive effect of device-guided paced breathing in diabetics: a randomized controlled study
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Background and Aims: Hypertension is a common comorbidity of diabetes. Slow breathing has been shown to reduce sympathetic activity and blood pressure (BP) in hypertensives. The objective of this study was to assess the efficacy of device-guided breathing in lowering blood pressure in diabetics with uncontrolled BP.
Materials and Methods: A randomized controlled study, carried out in urban family practice clinics in Israel. Non-insulin dependent diabetics with uncontrolled BP (>130/80 mmHg), receiving anti-hypertensive therapy or unmedicated, were enrolled. The intervention group used a device (RESPeRATE, InterCure Ltd, Israel), which interactively guides the user towards slow and regular breathing by synchronizing breathing voluntarily to musical tones, for 15 minutes daily at home for an 8-week period. Compliance and performance were monitored and analyzed from data automatically stored by the device during the treatment period. The control group continued with their regular treatment. The main outcome measure was the BP change, measured in the clinics at baseline, after 4 weeks and at termination at 8 weeks.
Results: Baseline characteristics of 60 patients (30 treatment and 30 controls), 36M/24F were: age 63±8 years (mean+SD); BMI 30±3 kg/m2; BP148±11/81±9 mmHg. The difference in BP change (mean+SE) between treatment and control groups was significant for systolic BP −9.5±1.9 vs.+2.1±2.4 mmHg, p<0.0005 for comparison between groups. Reduction of pulse pressure was significantly higher in the treatment group compared to control p=0.0007. Reduction of systolic BP correlated with the total time spent in slow breathing (less than 10 breaths per minute) during the 8 weeks of treatment, p=0.01.

BP value at start and end of study (mean+SE, mmHg)

	START	END	p value	START	END	p value	p value by group
	Treatment			Control			
Systolic BP	149.4±2.1	139.9±2.6	0.00002	146.2±1.8	148.3±3.0	0.38	0.0002
Diastolic BP	82.1±1.8	78.6±2.2	0.01	80.6±1.4	79.1±1.1	0.33	0.15
Pulse Pressure	67.3±2.5	61.4±2.5	0.01	65.6±2.1	69.2±3	0.14	0.0007

Conclusion: Self-treatment with device-guided breathing at home for 2 months by non-insulin dependent diabetic patients was associated with a significant reduction in office systolic BP and pulse pressure.

OP 38

Postprandial glucose regulation

0221

Is postprandial 2h glucose level a real determinant for glucose intolerance in people at risk for type 2 diabetes? RISK study results–I
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Background and Aims: To compare the efficacy of impaired fasting glycemia (IFG), impaired glucose tolerance at 1.h (IGT1) and impaired glucose tolerance at 2.h (IGT2) for the determination of glucose intolerance in population at risk for type 2 diabetes according to the ADA criteria.
Materials and Methods: The multicentric RISK study is population based and cross-sectional. It comprises 13.209 subjects (F/M: 1.75, mean chr. age: 52.95±11.5 years) randomly selected from 191 centres in Turkey. Inclusion criteria for the study were to be at a chr. age of ≥45 years or have risk factors for type 2 diabetes according to ADA criteria regardless of age. Family history of diabetes and for female subjects' history of gestational diabetes (GDM)

were interrogated. The examination included 75-g oral glucose tolerance test (OGTT), measurement of body mass index (BMI), blood pressure and lipid profile. Fasting blood glucose between 100–125 mg/dl and 1.h/2.h postprandial glucose levels between 140–199 mg/dl was considered as IFG, IGT1 and IGT2, respectively.

Results: IFG was diagnosed in 30.5% of the subjects. The OGTT revealed IGT1 in 41.4%, IGT2 in 23.9%, combined glucose intolerance (CGI: IFG+IGT) in 10% and diabetes (DM) in 3.3% of the subjects. Mean age was 53.68± 11.1 yrs. in IFG, 53.20±11.6 yrs. in IGT1, 53.8±11.2 yrs. in IGT, 54.12±10.7 yrs. in CGI and 54.74±10.8 yrs. in DM group. Female/Male ratio was 1.89 in IFG, 1.98 in IGT1, 1.84 in IGT, 1.88 in CGI and 1.89 in DM group. Ratio of positive family history of diabetes was 55.7% in IFG, 49.7% in IGT1, 56.9% in IGT, 58.6% in CGI and 67.2% in DM group. The prevalence of subjects with BMI>25 kg/m^2 was 84.2% in IFG, 84.8% in IGT1, 87.3% in IGT, 87.1% in CGI and 91.7% in DM group. Hypertension prevalence was 50.4% in IFG, 46.5% in IGT1, 53.9% in IGT, 57.4% in CGI and 64.3% in DM group.

Conclusion: In the evaluation of people at risk for type 2 diabetes according to ADA criteria, the rate of IGT according to WHO criteria was higher in IGT1(41.4%) than IGT2 (23.9%). If only 2.h glucose level was considered, 17.5% of people at risk of type 2 diabetes were missed, and with IFG 10.9%. These results show that fasting and 2.h glucose levels are not solely suggestive of glucose intolerance and evaluation of also the glucose values at the 1.h should be opened to discussion.

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0222

Effect of adjunctive mealtime pramlintide therapy on treatment satisfaction mediated primarily via attenuation of postprandial glucose excursions

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Background and Aims: In this study, subcutaneous (SC) pramlintide or placebo injections administered adjunctively with mealtime insulin in a cohort of intensively treated patients with type 1 diabetes (baseline A1C=8.1%) resulted in similar A1C reductions (−0.4 vs. −0.5%, p=0.6) after 29 wks of double-blind therapy. However, in pramlintide-treated patients, prandial insulin use decreased and the increase in basal insulin use was less than for placebo-treated patients (wk29%Δ from baseline: prandial −20 vs. +2%, p<0.01; basal+6 vs.+19%, p=0.08). Pramlintide-treated patients experienced superior postprandial glucose (PPG) control (average PPG: 151 vs. 172 mg/dl, p<0.01) and lost rather than gained weight (wk29 Δ from baseline: −1.5 vs.+1.3 kg, p<0.01). Responses on a 14-item study-specific treatment satisfaction (TS) survey completed under double-blind conditions also indicated pramlintide-treated patients were significantly more satisfied with the effects of study medication compared to PBO-treated controls. Given overall glucose control (A1C) was similar between the treatment groups, it was of interest to understand the relative contribution of other clinical endpoints in determining patients' reported treatment satisfaction. This post-hoc analysis evaluated the unique influence of 4-variables – including average PPG, A1C, weight and insulin use – on the aforementioned survey responses.

Materials and Methods: Intensively treated (CSII or MDI) patients with type 1 diabetes received double-blind SC mealtime injections of pramlintide (n=130) or PBO (n=136) for 29 wks. Mean baseline characteristics including A1C (8.1%), BMI (28 kg/m^2), duration diabetes (~20 yrs), and age (41 yrs) were similar across the groups. A 14-item TS survey was completed at wk29 under double-blind conditions. Items were rated on a 6-point Likert scale. Regression analysis was used to evaluate the independent associations of average PPG and wk-29 changes in weight, A1C, and total daily insulin dose with variation in TS. Analyses were performed for the combined study population and separately within each study group.

Results: After controlling for other clinical endpoints, average PPG was the strongest predictor of most (10/14) of the TS survey items for the combined study population, with lower (improved) PPG associated with higher TS. These relationships were stronger when the regression analysis was performed separately for the pramlintide-treated group and strongest for the following items: study medication made me feel better overall (F=16.0, p<0.01), outweighed the extra injections (F=15.4, p<0.01), increased flexibility in eating (F=12.9, p<0.01), made BG control more predictable (F=12.5, p<0.01) and provided more benefits than taking insulin alone (F=11.8, p<0.01); all partial correlation coefficients were ≥−0.35. In contrast, average PPG held little predictive utility within the PBO group.

Conclusion: Pramlintide-treated patients scored significantly higher than PBO-treated patients on the majority of items listed on a treatment satisfaction survey. In this study, the effect of pramlintide on attenuating PPG excursions was the primary factor differentiating the groups across survey items.

0223

Exenatide delays gastric emptying and reduces postprandial glucose in type 2 diabetes

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Background and Aims: Exenatide mimics several glucose-lowering actions of glucagon-like protein-1 (GLP-1). The effect of exenatide in type 2 diabetes may in part reflect delay of gastric emptying (GE). Exenatide slows acetaminophen absorption in healthy subjects and in patients with type 2 diabetes. This is the first study to directly quantify the effect of exenatide on GE by scintigraphy.
Materials and Methods: Seventeen patients with type 2 diabetes participated in a randomised, 3-period, single-blind, crossover study (16 males, 1 female; age, 39–69 yrs; BMI, 22.7–34.6 kg/m^2; HbA1c, 7.0–10.0%; treated with oral agents). Each patient was randomised to one of three treatment sequences containing all three treatments (PBO, 5 μg exenatide BID, and 10 μg exenatide BID). In the first 4 days of a 5-day period, patients received either 5 μg exenatide or placebo (PBO) subcutaneously (SC) BID, and patients in the 10 μg group received exenatide 5 μg SC BID for 2 days followed by 10 μg SC BID on days 3 and 4. Individual treatment periods were separated by a washout interval of at least 2.5 days. In all treatment groups, patients consumed a 450-kcal breakfast that included 99mTc-labeled eggs and 111In-labeled water immediately after the single morning dose on Day 5. Plasma exenatide, serum glucose and insulin, perceptions of appetite, and GE time were assessed for 6 hrs post-dose.
Results: Exenatide significantly delayed GE and reduced increases in postprandial glucose in a dose-dependent manner compared with PBO (see table below). Based on Visual Analogue Scales, exenatide had no significant effect on hunger, satiety, or fullness but significantly reduced prospective food consumption after the test meal compared with PBO (5 μg, P=0.03; 10 μg, P=0.07). The most frequent adverse event was mild-to-moderate nausea; no hypoglycemia occurred.
Conclusion: In patients with type 2 diabetes, exenatide treatment significantly delayed GE of solids and liquids and was associated with a significant reduction in postprandial glucose excursions.

	GE solids (T50, mins)	GE liquids (T50, mins)	Glucose*, C_{max} (mmol/L)	Glucose*, AUC (mmol x h/L)
PBO	59.6	33.9	13.3	56.8
Exenatide, 5 μg	111.3**	87.0**	10.9**	47.7**
Exenatide, 10 μg	169.4**	114.2**	9.7**	41.6**

Least Squares Geometric Means shown; *Mean premeal glucose concentrations (mmol/L): PBO, 10.3; 5 μg exenatide, 8.9; and 10 μg exenatide, 9.6. Premeal values adjusted for in statistical analysis. T50: half-emptying time. **P<0.01 vs. PBO.

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0224

Long-term treatment with exenatide improved postprandial glycaemic control and was associated with a shift from small to large HDL and LDL particles

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Background and Aims: Dyslipidemia in patients with type 2 diabetes is characterised by abnormalities in lipid concentration, lipoprotein size, and subclass composition. Recent studies have suggested that postprandial hyperglycemia is associated with vascular disease. The aim of this 52-wk study was to explore the effects of long-term exenatide treatment on dyslipidemia and postprandial glycaemia.
Materials and Methods: 52 patients participated in a test meal study which was part of a 1-year, open-label, phase 3 clinical trial comparing exenatide and biphasic insulin aspart 30/70 (n=505). Patients with type 2 diabetes inadequately treated with sulphonylurea and metformin were randomised to exenatide (n=30) or biphasic insulin aspart (n=22). For test meal data, we calculated postprandial glucose incremental $AUC_{0–4\ h}$ at baseline and Wk 52. Nontraditional cardiovascular (CV) markers, including precursor brain natriuretic protein, oxidized LDL-C, high-sensitivity C-reactive protein (hsCRP), fibrinogen, and apolipoproteins A1 and B, were assessed during fasting. Lipoprotein size and subclass composition were assessed using nuclear magnetic resonance technology.
Results:Both exenatide and biphasic insulin aspart treatment resulted in HbA_{1c} reductions (mean±SEM:

−1.4±0.2% and −0.8±0.2%, respectively). Postprandial glucose responses were similar at baseline (incremental $AUC_{0-4\ h}$: exenatide 4.4±1.1 mmol-h/L, biphasic insulin aspart 5.2±1.4 mmol-h/L). Both treatments significantly reduced postprandial glucose incremental $AUC_{0-4\ h}$ from baseline (exenatide −6.5±1.2 mmol-h/L, p<0.001; biphasic insulin aspart −5.4±1.6 mmol-h/L, p=.002). Body weight changes were −4.8±0.8 kg (p<0.001) for exenatide and +0.8±0.8 kg (p=.33) for biphasic insulin aspart. Exenatide-treated patients demonstrated a statistically significant reduction in fibrinogen from baseline (exenatide −0.45±0.22 g/L, p=.045; biphasic insulin aspart −0.24± 0.24, p=NS). No other statistically significant changes were observed at Wk 52 for the nontraditional CV markers; however, a trend toward reduction of hsCRP was observed (exenatide −0.62± 0.33 mg/L, p=.07; biphasic insulin aspart −0.06±0.40 mg/L, p=.87). Baseline to endpoint changes in lipoprotein particle size and concentration are shown in the table.

Conclusion: Exenatide and biphasic insulin aspart improved postprandial glycaemic control. Exenatide treatment was associated with reduced fibrinogen levels and a shift from small to large LDL and HDL particles. These findings suggest an improved cardiovascular risk profile after long-term treatment with exenatide.

Table: Lipoprotein Particle Size and Concentration

	Exenatide Change (mean, SEM)	Insulin Change (mean, SEM)	Between-Group Difference
LDL Particle Size (nm)	+0.33 (0.10) **p=.001**	+0.11 (0.12) p=.356	p=.144
HDL Particle Size (nm)	+0.11 (0.04) **p=.013**	+0.04 (0.05) p=.391	p=.318
VLDL Particle Size (nm)	−3.2 (1.9) p=.097	−1.6 (2.2) p=.482	p=.590
Total LDL (nmol/L)	−87.3 (62.2) p=.169	+5.0 (74.6) p=.947	p=.348
Large LDL (nmol/L)	+68.4 (27.3) **p=.017**	+37.7 (32.8) p=.258	p=.479
Small LDL (nmol/L)	−146.4 (67.7) **p=.037**	−25.8 (81.2) p=.753	p=.262
Very Small LDL (nmol/L)	−126.8 (53.9) **p=.024**	−17.0 (64.6) p=.794	p=.201
Total HDL (μmol/L)	+0.33 (0.79) p=.678	+2.5 (0.9) **p=.012**	p=.088
Large HDL (μmol/L)	+0.69 (0.33) **p=.042**	+1.0 (0.4) **p=.014**	p=.528
Total VLDL (nmol/L)	−9.3 (5.5) p=.097	+12.9 (6.6) p=.056	**p=.014**
Large VLDL (nmol/L)	−2.5 (1.6) p=.124	+0.13 (1.9) p=.945	p=.296

Supported by Eli Lilly and Company and Amylin Pharmaceuticals, Inc

OP 39

Optimising clinical diabetes care

0225

Costs of self-measurement of blood glucose (SMBG) regarding morbidity and mortality in type 2 diabetes in a reality of care setting (The ROSSO study No. 6)

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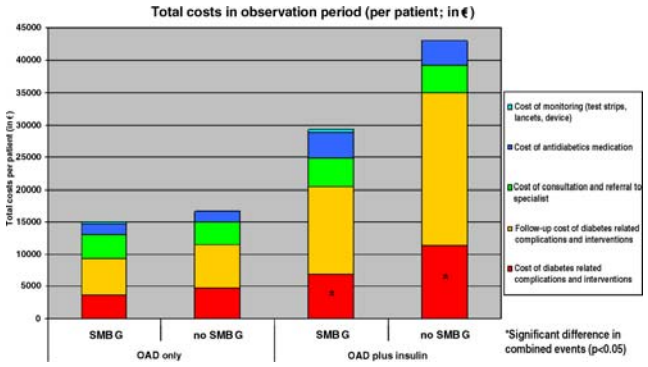
Background: A recently published large retrospective multi-centre study (RoSSO; Martin et al. Diabetologia 2006) showed in a German population that self measurement of blood glucose (SMBG) in patients with type 2 diabetes had a positive long-term outcome on hard endpoints. The aim of our analysis was to compare the costs of diabetes in the two study groups (patients with and without SMBG).

Methods: The original study groups (patients with and without SMBG) are in some points not comparable, because (e.g.) one group was in average 3.5 years older than the other. If adjustment for these differences was done by Cox regression in the original publication, adjustment for the present analysis had to be done by matching pairs based on age, gender, smoking status and blood glucose at diagnosis. In total 813 matched pairs (1626 patients) have been eligible for analysis. A further segmentation on the antidiabetic medication revealed 971 patients with oral antidiabetic drugs only ("OAD only") and 372 patients with oral antidiabetic drugs plus insulin ("OAD plus insulin"). Our assessment is based on costs for 18 diabetes related complications (including surgical interventions), follow-up-costs for these complications, costs for outpatient physician services, cost of antidiabetic and additional pharmaceutical treatment and costs for strips and lancets for patients performing SMBG using a German cost parameter set reflecting the situation in Germany in the year 2005.

Results: The following diagram summarizes the cost of type 2 diabetes and SMBG for patients treated with "OAD only" and patients with "OAD plus insulin". Use of SMBG in type 2 diabetic patients with "OAD only" leads to a non-significant cost saving of EUR 214 per patients and year, whereas a combined therapy "OAD plus insulin" causes significant cost savings of EUR 1'727 per patient and year.



Conclusion: Use of SMBG in type 2 diabetic patients may reduce complication related costs, whereby the costs for self-monitoring (test strips, devices) are compensated.

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0226

The implementation of a diabetes care system: a prospective observational 8-years follow-up study

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Background and Aims: In 1997, an extensive diabetes management programme, 'the Diabetes Care System (DCS)', was implemented in West-Friesland, The Netherlands. General practitioners (GPs) were invited to refer all their patients with diabetes to this care system, in addition to their care. The objective of the DCS was to provide the co-ordination of the sub-regional care including benchmarking of the main treatment outcomes, and to give feedback to the GPs. Additionally, to provide annual medical examinations for patients, and extensive education by diabetes nurses and dieticians in order to improve patient empowerment. At the start in 1997, 65 patients were included in the system. After a sharp increase of patients included during the first two years (n=803 in 1998 and n=1,330 in 1999), the average number of new patients every year was between 300 and 400. The aim of this study was to describe the changes in clinical characteristics of the patients after their entry into the system.

Material and Methods: A prospective observational cohort study of patients with diabetes who were included in the DCS was performed. From 1998 till 2005, data were collected every year on HbA_{1c}, weight, blood pressure, fasting blood glucose, total and HDL-cholesterol, triglycerides, medication and smoking status. To evaluate individual patients characteristics following entry into the care system, a study cohort was defined. In this cohort, for every individual patient, the measurements in their first year of entry into the system were set at T=1. Follow-up measurements were defined as T=2, for the second year, to T=8, for the eight year after entry into the system.

Results: The defined study cohort started with 4,933 patients at T=1. Mean age of the patients was 62 years (SD±12 years) and 51% was male. Body mass index was 29.8 kg/m^2 and did not change during follow-up. Glycaemic control showed an initial sharp decrease, followed by stabilization during follow-up. The use of insulin therapy and diabetes duration increased. Total cholesterol and triglycerides decreased during the 8 years of follow-up whereas HDL-cholesterol increased. Systolic blood pressure increased, but diastolic blood pressure decreased. The percentage of smoking patients decreased from 21% at T=1 to 15% at T=8.

Changes in clinical characteristics during a prospective observational 8-years follow-up study

	T1	T2	T3	T4	T5	T6	T7	T8
Number of patients	4,933	3,709	3,179	2,539	1,974	1,526	1,049	461
Diabetes duration (years)	4.6 (6.1)	5.5 (5.7)	6.7 (5.7)	7.7 (5.6)	8.8 (5.5)	10.1 (5.8)	11.5 (5.8)	13.4 (5.7)
HbA_{1c} (%)	7.7 (1.8)	7.0 (1.2)	7.1 (1.2)	7.2 (1.2)	7.1 (1.2)	7.2 (1.2)	7.1 (1.1)	7.1 (1.1)
Systolic BP (mmHg)	143.3 (21.8)	141.6 (21.7)	142.4 (21.6)	144.1 (21.7)	147.3 (21.5)	149.4 (22.2)	151.3 (21.7)	151.6 (21.3)
Diastolic BP (mmHg)	82.6 (11.3)	81.3 (11.1)	80.9 (11.3)	80.5 (10.8)	79.7 (10.9)	78.6 (10.5)	77.9 (10.0)	76.4 (10.3)
Fasting blood glucose (mmol/l)	8.9 (3.5)	8.2 (2.2)	8.3 (3.0)	8.3 (2.5)	8.2 (2.9)	8.3 (2.2)	8.2 (2.1)	8.2 (2.3)
Total cholesterol (mmol/l)	5.4 (1.1)	5.3 (1.1)	5.2 (1.1)	5.2 (1.1)	5.1 (1.0)	5.1 (1.1)	4.9 (1.0)	4.8 (1.0)
HDL-cholesterol (mmol/l)	1.2 (0.4)	1.3 (0.4)	1.3 (0.3)	1.3 (0.4)	1.3 (0.4)	1.3 (0.4)	1.3 (0.4)	1.4 (0.4)
Triglycerides (mmol/l)	2.1 (2.0)	1.9 (1.2)	1.9 (1.2)	1.9 (1.1)	1.8 (1.0)	1.8 (1.1)	1.8 (1.1)	1.8 (1.0)
Patients on insulin (%)	12	17	20	24	28	32	35	41

Data are means (standard deviation) or %

Conclusion: The Diabetes Care System was successful in improving and stabilizing the clinical characteristics for individual patients, despite the longer mean diabetes duration. This implies that a disease management system

may be a major step in the improvement of the care of patients with diabetes.

0227

Cost-effectiveness of an intensified multi-factorial intervention vs. conventional treatment in microalbuminuric patients with type 2 diabetes at a high CVD risk: results from the Steno-2 study

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Background and Aims: The Steno-2 study was a long-term, randomised, open, parallel trial that compared an intensified, multitargeted intervention versus conventional multifactorial treatment in patients with type 2 diabetes and microalbuminuria. Patients received conventional treatment in accordance with national guidelines, or an intensified, integrated treatment targeting glycaemia, lipid and blood pressure abnormalities, microalbuminuria and platelets. The primary composite endpoint was cardiovascular disease (CVD) i.e. non-fatal MI, non-fatal stroke, PCI, coronary artery bypass grafting, revascularisation of the leg, amputation and CVD death. After 7.8 yrs, patients in the intensive arm had a 53% (95% CI: 27–76) lower relative risk of CVD. The aim of the current study was to assess the cost-effectiveness of intensive versus conventional therapy as applied in the Steno 2 study from the perspective of a Danish reimbursement authority.
Materials and Methods: A mathematical simulation Markov model was developed which incorporated annual CVD events, heart failure-related hospital admissions, dialysis and total mortality rates that were capture during the Steno 2 study. Danish-specific event- and intensive/conventional intervention direct medical costs were extracted from Danish cost databases by local experts. An 8-year analysis (approximating the with-in trial period), as well as longer-term projections were made. Outcomes that were calculated included: quality-adjusted life expectancy (in quality–adjusted life years [QALYs]), mean total costs per patient, and incremental costs per QALY gained. Future costs and QALYs were discounted in line with current international guidelines (3% annually) to account for time preferences for money and good health. Extensive sensitivity analyses were performed to assess the robustness of the findings.
Results: Intensive intervention led to substantial improvements in QALY versus conventional intervention. The higher costs of implementing the intensive therapy were partially offset by reduced costs of complications. Projections over longer time horizons led to greater improvements in QALYs and smaller differences in costs between the treatment arms. Sensitivity analysis revealed that if the intensified therapy could be implemented as effectively in a primary care setting, it would be cost saving compared to conventional therapy.
Conclusion: A multifactorial intensified intervention is highly cost-effective compared to conventional therapy in a Danish setting by current international standards. If the intensified intervention can be implemented as effectively in a primary care setting, substantial cost savings to the Danish healthcare system would be anticipated.

Summary of Economic Outcomes for Intensive versus Conventional Therapy

	Time Horizon					
	8-Years (approximating with-in trial period)	8-Years (approximating with-in trial period)	10-Years	10-Years	30-Years	30-Years
	Costs (Danish Kroner)	QALY (Years)	Costs (Danish Kroner)	QALY (Years)	Costs (Danish Kroner)	QALY (Years)
Intensive	161,059	5.50	191,794	6.54	339,170	10.72
Conventional	127,610	5.34	160,531	6.27	308,420	9.41
Difference	33,449	0.16	31,263	0.28	30,750	1.31
Costs/QALY gained	211,702	–	113,271	–	23,366	–
Costs/QALY gained (€)	28,377	–	15,183	–	3,132	–

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0228

The influence of an intensive treatment and teaching programme on the progression of diabetes late complications in type 1 diabetic patients in Moscow (15-year results)

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Background and Aims: Patient education and self-monitoring is regarded as the basis for a reduction of diabetes-related acute and late complications, improvement of diabetes care and saving of health care cost. The aim of

the study was to evaluate metabolic control and progression of late diabetes complications after structured programme for intensive treatment and teaching of Type 1 diabetic patients (DTTP) during long-term follow-up.
Materials and Methods: 65 Type 1 diabetic patients (28 male, 37 female) were re-examined after DTTP during follow-up period of 15 yrs. The mean age at the end of study was 43,6±8,6 (30–62) yrs; mean diabetes duration 25,8±7,4 (15–46) yrs. Patients were divided two subgroups (SG I, SG II). SG I (n=24) was followed–up in study center, these visits included elements of individual education (review and adjustment regimens of insulin therapy, improvement knowledge and skills, discussion urgent problems and etc.). Patients of SG II (n=41) were treated in system of public health without intensive follow–up. All results of this study were compared with data before DTTP. To evaluate progression of diabetic retinopathy fundus photos were taken before DTTP and during follow–up period. For detection of diabetic nephropathy we measured albumin excretion rate and glomerular filtration rate.
Results: There was significant improvement of HbA1c 1 yr after DTTP (from 9,5±0,9% to 7,5±1,0%, p<0,001), SG I did not differ from SG II. HbA1c increased to 8,4±1,7% (p<0,005 compared initial level) 7 yrs after and to 8,9±1,8% (p<0,01) 15 yrs after DTTP. HbA1c was significantly lower in SG I compared with SG II: 7,7±1,1% vs 8,8±1,8% (p<0,001) in 7 yrs and 7,9±1,1% vs 9,3±1,9% (p<0,001) in 15 yrs. There was significance difference in blood glucose self–monitoring frequency between subgroups 15 yrs after DTTP: 26,1±17,7 in SG I vs 10,3±9,5 per week in SG II (p<0,001). 40% patients had diabetic retinopathy before DTTP, 72% and 100% patients had such late complication 7 and 15 yrs after. The prevalence of proliferative retinopathy during follow–up increased from 1,5% before to 12,3% 7 yrs and 20,0% 15 yrs after DTTP, but there was significant difference between SG I and SG II in 15 yrs: 8,3% vs 26,8% (p<0,05). The prevalence of diabetic nephropathy did not increased. 20% patients had micro-albuminuria initially, 7,7% – in 7 yrs, 15,4% – in 15 yrs (p>0,05). Proteinuria was found in 4,6% patients before DTTP, in 12,3% 7 yrs after, in 9,2% 15 yrs after (p>0,05). The prevalence of all stages of nephropathy was similar in both subgroups.
Conclusion: Type 1 diabetic patients had significantly better level of glycemic control during 15 yrs follow-up compared with baseline before DTTP. Also this study has demonstrated deterioration of metabolic control during long-term follow-up after DTTP. But intensive follow-up after DTTP can help to achieve better HbA1c level compared with routine diabetes care in public health system. This study has shown effectiveness of DTTP and long-term intensive follow-up in respect of late diabetes complications.

OP 40

Coronary endothelial function

0229

Coronary vasoreactivity is not altered in young uncomplicated type 1 diabetes

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Background and Aims: Abnormalities of coronary vasoreactivity have been reported in diabetic patients even in the absence of coronary artery disease. How early these abnormalities occur in Type 1 diabetes is still unsettled.
Materials and Methods: We evaluated coronary microcirculation function in thirty-one Type 1 diabetic patients (D) (age 31±8 y, BMI 24±3 kg/m2, duration of diabetes 11±8 y, HbA1c 7.9±1.0%) and in 11 normal subjects (N) (age 32±7 y, BMI 23±2 Kg/m2). The two groups were similar for blood pressure and lipid profile, and no subject had a positive family history of coronary heart disease. Diabetic patients were free of macrovascular complications, had a normal exercise treadmill test and only few of them had initial microvascular complications (6/31 showed signs of background retinopathy and 7/31 microalbuminuria). Blood flow velocity in the left anterior descending coronary artery was measured at rest and after dipyridamole (0,56 mg/kg for 4 min followed by 0,26 mg/Kg for 2 min) using transthoracic color-guided pulsed Doppler echocardiography. Coronary flow reserve (CFR) was defined as the ratio of dipyridamole-induced coronary peak flow velocity and resting flow velocity. CFR assessment was performed in the fasting state before administration of the morning short-acting insulin dose.
Results: Both systolic and diastolic blood pressure were similar in D (119±10/74±8 mmHg) and C (121±24/78±8 mmHg) and remained substantially unchanged during dipyridamole infusion. Heart rate at rest was 72±12 in D and 73±12 beats/min in N and increased by 30–40% after dipyridamole in both groups. Coronary flow velocity was 23±0.7 in D and 24±0.5 cm/sec in N at rest and increased to 69±1.6 cm/sec and 77±1.7 cm/sec in D and N, respectively (p=n.s.) CFR was similar in the two groups (3.15±0.7 and 3.20±0.7 in D and N). No correlation was found between

CFR and duration of the disease or degree of glycemic control. All indexes of both diastolic and systolic function were similar in D and N.
Conclusion: Coronary vasoreactivity and myocardial function are well preserved in young uncomplicated Type 1 diabetic patients. This observation suggests that in the absence of vardiovascular risk factors, coronary vasoreactivity impairs later on in the course of type 1 diabetes.

0230

Coronary endothelial dysfunction and arterial stiffness, but not microalbuminuria, are of poor cardiovascular prognosis in diabetics without other cardiovascular risk factor

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Background and Aims: An association between peripheral endothelium dysfunction, microalbuminuria (MA) and arterial stiffness has been suggested. MA has been shown to be a powerful predictor of cardiovascular events (CVE) in diabetic patients. The aim of the study was to evaluate the relation between coronary endothelium dysfunction (CED), MA and arterial stiffness and their respective prognostic value in patients with type 2 diabetes (T2D) but no other major CV risk factor.
Patients and Methods: A total of 84 patients with T2D for 9.3±2.7 years, with atypical cardiac symptoms and with positive or equivocal ECG stress test or myocardial scintigraphy but angiographically normal coronary arteries were included. They were aged <65 yrs, normotensive, non smokers, non obese, and had LDL cholesterol <3.7 mmol/l. CED was evaluated according to the diameter changes of the left anterior descending coronary artery (ΔDC) after a cold pressor testing during a quantitative coronary angiography. Arterial stiffness was estimated by the brachial pulse pressure. The patients were followed up for CVE (cardiac death, myocardial infarction, stable or unstable angina, coronary artery revascularization, stroke).
Results: A CED, defined as a decrease in coronary artery diameter>6%, was present in 62 (74%) patients. MA was present in 25 patients and urinary albumin excretion rate correlated with ΔDC ($r=-0.39$, $p=0.003$) and pulse pressure ($r=0.244$, $p<0.05$). A CVE occurred in 19 patients, after 45±19 months of follow-up. The predictive factors for CVE were CED (29% vs 4.5%, $p<0.01$), higher systolic blood pressure ($p<0.05$) and pulse pressure ($p<0.05$). The risk associated with CED was unmodified by the presence of MA (CED− MA−: 5.6% of CVE, CED− MA+: 0%, CED+MA−: 31.7%, CED+MA+: 23.8%).
Conclusions: These data suggest that 1) CED and arterial stiffness are predictive of early CVE in the T2D population without other CV risk factor ; 2) MA is a marker of CED and arterial stiffness. 3) CED and arterial stiffness might account for the increased CV risk linked to MA.

0231

Pioglitazone improves insulin sensitivity and endothelial function in coronary artery disease patients with unknown impaired glucose tolerance

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Objective: Diabetes is often undiagnosed and untreated in patients suffering from coronary heart disease (CHD). However, a medical history characterized by **c**oncurrence of previous Acute Myocardial Infarction (AMI) and Type 2 diabetes (T2DM) is known to identify patients at the highest risk for cardiovascular mortality, due to recurrent AMI events. Moreover, activation of inflammatory pathways often is present in patients with unknown diabetes and in CHD patients with tendency to recurrent events. The aims of this study were 1) to evaluate the prevalence of glucose metabolism disturbances in CHD patients; 2) to correlate the glucose metabolism status with inflammatory markers; and 3) to identify in a pilot study a reasonable therapeutic approach acting to dampen both impaired glucose metabolism and inflammation.
Methods: To evaluate the prevalence of glucose metabolism disturbances in CHD patients, we performed an oral glucose tolerance test (OGTT) in a large group of patients with a previous MI and without known T2DM, no differences in secondary prevention treatments. In all patients we also evaluate inflammatory markers (hsCRP, TNF-alpha, CD40L and adiponectin). Then, we tested the short-term effect of pioglitazone (n=12) compared to placebo (n=11) on inflammation state, insulin sensitivity (euglycemic hyperinsulinemic clamp, IS) in a sub-group of patients affected by impaired glucose tolerance (IGT) after the OGTT.
Results: In 185 non T2DM CHD patients all matched for age, sex, waist, BMI, smoking and pharmacological treatments, upon OGTT, we observed that 21% patients were affected by T2DM, 38% by IGT, and only 41% were

normoglycemic (NGT). Among the inflammation markers (compared with univariate ANOVA) we observed that only TNF-alpha was significantly higher in both IGT and T2DM compared to NGT patients ($p<0.01$). Interestingly, adiponectin was higher in NGT compared to the other two groups ($p<0.05$). In overall patients, a stepwise logistic regression analysis showed that TNF-alpha was the main predictor for defects of glucose tolerance ($p<0.01$). Next, 23 patients from the IGT group were randomized to receive pioglitazone (30 mg/die, 12 patients) or placebo (11 patients) for 12 weeks. At the end of treatment we observed that pioglitazone significantly reduced fasting insulin (but not fasting glucose) as well as TNF-alpha levels, when compared to the placebo group. Remarkably, we observed that Pioglitazone increased adiponectin and IS, assessed by a hyperinsulinemic euglycemic Clamp compared to the placebo group ($p<0.05$ for all).

Conclusions: Our study suggests that an unchecked inflammatory state characterized by increased TNF-alpha and reduced adiponectin levels in patients with a prior AMI is associated to disturbances of glucose metabolism. Pioglitazone can impede progression to diabetes and reduce inflammatory status. Since previous studies separately observed that increased TNF-alpha and undiagnosed diabetes in CHD patients were associated to higher mortality due to recurrent coronary events and/or heart failure, our study suggest that all CHD subjects must be evaluated through OGTT to adequately treat IGT and T2DM.

0232

Exendin-4 rapidly activates eNOS and protects human coronary artery endothelial cells against glucolipoapoptosis

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Background and Aims: Endothelial dysfunction plays an important role in macrovascular complications in diabetes. We recently showed that the glucagon-like peptide-1 receptor is expressed in human coronary artery endothelial cells (HCAEC) and that GLP-1 improves endothelial dysfunction in type 2 diabetic patients with coronary artery disease. The aim of the present study was to investigate the role of the stable GLP-1 receptor agonist exendin-4 on neogenesis and apoptosis under normal and hyperglucolipidemic conditions (simulating the diabetic milieu) in HCAEC cells *in vitro*. Exendin-4 was approved for clinical use in the U.S. in 2005 against type 2 diabetes.

Materials and Methods: HCAEC cells were cultured in the endothelial growth medium in the presence of 5 mM glucose. Phosphorylation and expression of endothelial nitric oxide synthase (eNOS) were examined by Western blotting using anti-phospho-eNOS and anti-eNOS antibodies, respectively. β-actin examined in same blots acted as loading control. The concentration of NO in the culture medium was determined by the Saltzman assay. Results were normalized by subtracting the NO content in the wells with medium alone in the incubation. ^{3}H-thymidine incorporation was assayed in 96-well plates as a measure of DNA synthesis, and caspase-3 activity was evaluated using ELISA kit as a measure of apoptosis. DNA synthesis and apoptosis were examined after 48 h-incubation with the reagents. Cell death was assessed using Trypan-blue inclusion.

Results: Incubation of HCAEC cells with exendin-4 from 1 to 10 nM for 10 min resulted in a dose-dependent increase of eNOS phosphorylation to an extent comparable with that of GLP-1 and acetylcholine. Continuous treatment of the cells with exendin-4 for 48 h resulted in increased expression of eNOS protein. Similar to the acute effects of the peptide, long-term treatment with exendin-4 also enhanced the phosphorylation state of the enzyme even when normalized for eNOS expression. Incubating cells in 30 mM glucose resulted in decreased NO production, an effect normalized by co-incubation with exendin-4. In contrast, palmitate-mediated NO production was not affected by this treatment. Exendin-4 promoted HCAEC DNA synthesis after 48 h incubation in 5 mM glucose, and neogenesis was confirmed by an increased cell number. Incubation of the cells with high glucose or palmitate provoked apoptosis and cell death, both of which were completely prevented by exendin-4.

Conclusions: Our study reveals salutary effects of exendin-4 on cell regeneration and protection from glucolipoapoptosis in human coronary artery endothelial cells, These novel findings add yet other beneficial properties of exendin-4, increasing its clinical utility in type 2 diabetic patients in whom endothelial dysfunction is a salient feature that adversely affect their survival. Elucidation of the mechanisms underlying these beneficial vascular effects of exendin-4 may be exploited when intervening pharmacologically against vascular dysfunction in diabetes and may form the basis of improved incretin enhancers that hold the potential to prevent vascular lesions in diabetic patients.

OP 41

Prediction and prevention of type 1 diabetes

0233

Serum metabolite patterns between birth and development of autoantibodies and overt type 1 diabetes: application of large-scale metabolomics to the Type 1 Diabetes Prediction and Prevention study (DIPP)

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Background and Aims: To explore etiopathogenesis and prevention possibilities of Type 1 diabetes (T1D), over 8000 children carrying HLA-conferred genetic risk for T1D have been followed since birth at 3- to 12-month intervals for development of T1D-related autoantibodies (ICA, IAA, GADA and IA-2A) and overt T1D in Type 1 Diabetes Prediction and Prevention Study (DIPP), launched in Finland in 1994. We continue screening of over 10,000 newborn babies annually for HLA-conferred T1D risk to enroll those at risk in the study. As changes in groups of metabolites may be descriptive of systemic responses to genetic or environmental exposures, they may function as a powerful tool for characterization of complex phenotypes and as biomarkers for specific physiological responses. We thus hypothesized that extended serum metabolite patterns might differ between children remaining autoantibody negative (controls) and children who later develop autoantibodies and progress to T1D (cases), and that the changes might reflect etiopathogenetic events that by far precede development of autoimmunity. Thus, our aim was to elucidate these early events leading to autoimmunity and overt T1D by analyzing our 11.5-year collection of longitudinal serum sample series from the DIPP case and control children.
Materials and Methods: DIPP serum samples were stored at −70°C. The metabolite profiles of the sample series collected from 46 cases (progressed to T1D) were compared with the series collected from 59 controls (remained autoantibody negative) matched for time of birth, gender, genetic T1D risk, and city of birth (total 1234 samples). Ultra Performance Liquid Chromatography coupled to high resolution mass spectrometry (MS) and gas chromatography coupled to mass spectrometry were used. Data were processed with MZmine software, and metabolites identified using in-house databanks and tandem MS.
Results: Metabolite profiles and identified key metabolites separate the cases from the controls much before autoantibodies emerge. The changes reflect alterations in gut permeability, antioxidant capacity and inflammation.
Conclusion: Our data suggest that early metabolic insult(s) may lead to alterations in the immune system making it autoimmunity-prone. The pre-seroconversion changes support the trigger-booster hypothesis claiming that the processes leading to T1D are triggered by an exogenous factor driven by one or several environmental determinants. Our data further suggest that advanced high-throughput metabolomics methods may markedly antedate and improve accuracy of defining which children will later progress to autoimmunity and T1D.
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0234

Elevated IL-4 levels at birth may predict type 1 diabetes

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Background: Reliable prediction is a prerequisite for developing and testing prevention strategies for type 1 diabetes (T1D). Beta cell destruction in T1D pathogenesis is thought to be mediated by an immune response against the beta cells involving pro-inflammatory T helper cell type 1(Th1) cytokines (IL-1b, IFNg, TNFa) from islet-infiltrating T cells, Natural Killer (NK) cells and macrophages, whereas anti-inflammatory Th2 cytokines such as IL-4 and IL-10 may protect against T1D. At time of birth, predominantly Th2 immune responses are present in healthy individuals. Th1 levels are increasing during childhood. It is still unknown if cytokine levels differ in newborns who later in life develop T1D as compared to non-diabetic controls.

Aims: To determine levels of cytokines and other inflammatory markers at time of birth in a large unselected population-based case-control population of newborns using Dried Blood Spots (DBS) at day 5 and test the hypothesis that levels of inflammatory markers at birth predict T1D.
Materials: DBS of 2086 Danish T1D patients from the birth cohorts of 1981–2002, and 2 controls per patient were selected. Patient and control samples were matched by place and date of birth. The samples were stored for 3–24 years at −25° at the Statens Serum Institut, Copenhagen, Denmark.
Methods: Cytokines (IL-1b, IFNg, TNFa, TGFb, IL-4, IL-6, IL-8, IL-10, IL-12), MBL, CrP, TREM-1, Adiponectin and Leptin were measured by use of the flowmetric Luminex® xMAP technology. Samples were analyzed simultaneously for all markers. Analysis has been performed and evaluated in the first 2780 samples. Cases and matched controls were run together. GAD65 (GADA) and IA-2 (IA-2A) autoantibodies in combination was measured by standard radiobinding assay on DBS eluates. Conditional logistic regression was used to estimate hazard ratios of diabetes occurrence from the matched case control data.
Results: Proportional Hazard Regression model in patients compared to their matched controls showed a Hazard Ratio (HR) of 1.108 (confidence interval (CI) 1.034–1.186, $p<0.0035$) and 1.402 (CI 1.033–1.901, $p<0.03$) for every increase with 10 pg/ml of IL-4 and for every 10-fold increase of IL-4 (=log10), respectively. HR for possibly interacting cytokines was 1.136 (CI 1.024–1.261, $p<0.017$) and 1.299 (CI 1.044–1.616, $p<0.019$) for log10 (IL-4*IL-10) and log10 (IL-4*IL-12), respectively. No differences were detected between cases and controls for any of the other inflammatory markers. In cases positive for GADA, IA-2A, or both, HR for log10 (IL-4) was 5.475 (CI 2.841–10.55, $p<0.0001$), compared to HR=1.366 (CI 1.001–1.863, $p<0.049$) for log10 (IL-4) in patients compared to controls negative for GADA and IA-2A.
Conclusion: Elevated IL-4 levels at the time of birth may be a prediction marker of T1D diagnosed before 24 years of age, particularly in individuals positive for islet autoantibodies against GAD65, IA-2, or both.
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0235

Islet antibodies at birth predict development of type 1 diabetes

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Background: It is still unknown if diabetes-related autoantibodies at the time of birth impact the development of type 1 diabetes (T1D). A reason for current controversies is the lack of sufficiently large investigations at time of birth of subjects who later developed T1D. Despite considerable efforts current prospective studies ascertain only a limited number of new patients per year. Other complicating issues affecting statistical power include transplacental transfer of autoantibodies and their disappearance during the first year of life, and the fact that studies such as BABY DIAB focus on children with first degree relatives with T1D, despite only 15% of new onset patients belong to this category. Population-based investigations would therefore seem to be a requisite to establish whether islet autoantibodies at birth predict T1D.
Aims: To determine GAD65 and IA-2 autoantibodies at time of birth in a large unselected population-based case-control population of newborns using Dried Blood Spot (DBS) collected 5 days after birth. We test the hypothesis that islet autantibodies at birth predict T1D.
Materials: DBS of 2028 Danish T1D patients from the birth cohorts of 1981–2002, and 2 controls per patient were selected. Patient and control samples were matched by place and date of birth. The samples were stored for 3–24 years at −25° at the Statens Serum Institut, Copenhagen, Denmark. Gender, age of gestation, birth weight (BW), birth length (BL), age at clinical diagnosis of T1D, and mothers' potential diabetes diagnoses were known.
Methods: A radiobinding assay was performed on 3.2 diameter DBS punch-out samples, simultaneously detecting autoantibodies to GAD65, IA-2, or both. Autoantibody positive samples (by 99% percentile) are re-analyzed for IA-2 autoantibodies alone to distinguish the possible role of GAD65 from IA-2 autoantibodies.
Results: In the case group, 70/2028 (3.45%) were autoantibody positive compared to 21/4021 (0.52%) in the control group ($p<0.0001$). The 99.5% percentile in the control population was used as a cut-off point. Proportional Hazard Regression Model in cases compared to their

matched controls showed a Hazard Ratio of 2.54 (confidence limits 2.002–3.242) for every 10-fold increase in relative units (an expression of autoantibody levels) ($p<0.0001$). Antibody positive compared to negative patients did not differ with respect to age at diabetes diagnosis, birth weight, birth length, or gestational age (determined by regression analysis). Only 2/70 (2.86%) autoantibody positive cases were born to mothers with known diabetes (T1D). A total of 88/1958 (4.49%) autoantibody negative cases were born to mothers known to have any form for diabetes (1.74–2.24% T1D (0.5% not specified), 1.63% T2D, 0.7% gestational diabetes).

Conclusion: Detection of islet autoantibodies against GAD65 and IA-2 at the time of birth is a significant prediction marker of T1D diagnosed before 24 years of age.

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0236

The Swedish GAD-vaccination Trial: outcomes of a phase II safety and efficacy trial with Diamyd™ for preservation of beta cell function in children with T1D

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Background and Aims: Numerous studies suggest Glutamic Acid Decarboxylase (GAD65) is a major autoantigen in type 1 diabetes. Evidence has recently been reported for the preservation of beta cell function by vaccination with alum-formulated rhGAD65 (DiamydTM) in adults with slowly progressive autoimmune diabetes (LADA). To investigate if GAD-treatment is safe and can preserve beta cell function in patients with Type 1 diabetes (T1D) – which is considered as a more aggressive form of autoimmune diabetes than in LADA – a phase II randomised, double blind, and placebo controlled trial has been conducted in 70 T1D patients.

Materials and Methods: The study was approved by The Regulatory Authorities in Uppsala, Sweden and by the Ethic's Committee in Linkoping, Sweden. The study was carried out at eight centers in Sweden. Informed consent was granted by patients and their parents.

118 patients between 10–18 years of age and diagnosed with T1D within 18 months were screened before 70 patients were found to meet the two main inclusion criteria: GADA titer>23 WHO U/mL and a residual fasting C-peptide level of at least 0.1 nmol/l. Patients were randomised into 2 groups with 35 patients receiving 20 ug DiamydTM at day 1 and 28, and 35 patients assigned to the placebo arm receiving the same formulation without rhGAD65. Patients revisited at 3,6,9 and 15 months at which point metabolic and immunologic parameters were measured. C-peptide levels before and after Sustacal challenge were measured as a surrogate for beta cell function.

Patients are stratified for several parameters including HLA, age, gender, disease duration, GADA, HbA1C and C-peptide levels at inclusion.

Results: One patient developed a serious adverse event after having received one of the two intended injections, and did not receive the second injection. The treatment code was broken showing that the patient had been allocated to the placebo group. No other Serious Adverse Reactions have been reported. Clinical outcomes regarding safety and efficacy, together with the results from analyses of impact of treatment on parameters reflecting immunologic and beta cell function, will be reported from this first clinical trial with DiamydTM in patients with recent-onset T1D.

Conclusion: Treatment of children with recent onset T1D with 20 ug DiamydTM seems safe and feasible. Impact of treatment on parameters reflecting immunologic and beta cell function will be reported.

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OP 42

Pancreas and islet transplantation

0237

Islet-graft response to transplantation injury includes up-regulation of protective as well as apoptotic genes

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Background and Aims: Around 60% of pancreatic islet tissue is lost within the first days after transplantation. Islet damage is multifactorial and includes an early non-specific inflammatory response that takes place before rejection or autoimmunity recurrence. We have recently shown that the expression of IL-1β, IL-6, IL-10 and TNF-α genes is increased early after islet transplantation, suggesting their contribution to the initial graft loss. Here, we investigated the molecular mechanisms involved in beta cell damage and survival after pancreatic islet transplantation. We studied the expression of a set of selected genes involved in apoptosis (bcl-2, bax, bad, bid and C/EBP homologous protein [CHOP]), cytokine defense, (suppressor of cytokine signaling [socs]-1 and socs-3) or free radical protection (heme oxygenase-1 [HO-1], Cu/Zn-superoxide dismutase [SOD], Mn-sod and hsp70). Since hyperglycemia has deleterious effects on islet transplantation outcome, we studied its effect on the expression of these genes.
Materials and Methods: 500 islets were syngeneically transplanted under the kidney capsule of normoglycemic or streptozotocin-induced diabetic Lewis rats. Gene expression was analyzed by quantitative real-time RT-PCR in grafts harvested 1, 3 and 7 days after transplantation, and in freshly isolated islets.
Results: The expression of protective genes socs-1, socs-3, HO-1 and Mn-sod as well as that of pro-apoptotic genes bid and CHOP was maximally increased one day after transplantation, and in most cases it remained increased, although at a lower level, seven days later. In contrast, the expression of bcl-2, bax, bad, Cu-sod and hsp70 genes was unchanged. No differences were found between grafts transplanted to normoglycemic and hyperglycemic recipients. SOCS-1 and SOCS-3 negatively regulate cytokine signaling, and HO-1 expression increases in beta cells exposed to TNF-α or IL-1β contributing to the anti-inflammatory response of islets. Altogether, the increased expression of SOCS-1, SOCS-3 and HO-1 supports the involvement of pro-inflammatory cytokines in the early inflammatory response in islet grafts. The expression of bid may be related to anoikis and to the extrinsic apoptotic pathway, linking membrane death signaling to mitochondria. The expression of mitochondrial antioxidant gene Mn-sod, but not that of cytosolic Cu-sod, was increased suggesting a role of mitochondrial free radicals in islet damage. The increased expression of CHOP gene points to the occurrence of severe ER-stress, which has been recently linked to beta cell death.
Conclusion: Our results suggest an important role of inflammatory cytokines and free radical formation in islet graft damage early after transplantation. Anoikis can also be relevant as suggested by the increased bid expression. ER-stress and mitochondrial dysfunction are early events in islet graft dysfunction. In contrast, hyperglycemia may not be a major islet insult in the immediate post-transplantation period.
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0238

Metabolic effect of sirolimus versus mycophenolate mofetil on pancreatic graft function – a prospective randomised study

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Background: Metabolic effects of immunosuppressive agents are of great importance in pancreas or islet transplantation. The aim of our study was to compare the glucose metabolism in Type 1 diabetic recipients of kidney and pancreatic grafts on tacrolimus-based immunosuppression in conjunction with sirolimus (RAPA) or mycophenolate mofetil (MMF) in a prospective randomised study.
Methods: The investigation was performed in 26 insulin-independent rejection-free patients after simultaneous pancreas and kidney transplantation (with systemic venous drainage of pancreatic graft) at 2 occasions: at 0.5±0.2 [mean±SD] month post-transplant (with steroid dose 12.1±4.0 mg/day) and subsequently at 24.2±10.7 months (steroid-free). All recipients had a good function of the kidney graft (serum creatinine level 114.0±26.1 μmol/L). Fasting glycemia, insulin and C-peptide levels, HbA_{1c}, standard intravenous glucose tolerance test (IVGTT) with coefficient of glucose assimilation (K_G) calculation were assessed in both groups. Insulin sensitivity was evaluated by the homeostasis model assessment (HOMA–IR). Areas under the insulin/C-peptide curves during the IVGTT (AUC-IRI, AUC-CP) were used as the parameters of insulin/C-peptide secretion.
Results: The RAPA and MMF groups did not differ in age, post-transplant period and steroid daily dose. The results (with differences between 1st and 2nd examinations in single groups) are shown in the table. However, we did not find any significant difference between the groups in main parameters. The only difference between RAPA and MMF groups was lower stimulated C-peptide level in the first mentioned one (Examination 2: IVGTT – Minute 6: 1.3±0.54 vs. 1.9±0.78 pmol/mL, $p<0.05$). Trough levels of tacrolimus (in therapeutic range) had no significant impact on any of examined parameters. The response of IVGTT (K_G) and HOMA–IR of whole study group significantly improved

between the exams (1.03±0.43 vs. 1.4±0.51, p<0.01 and 3.49±2.39 vs. 2.47±1.77, p<0.05, respectively).

Conclusion: Glucose tolerance measured with the use of IVGTT significantly improved due to the increased insulin sensitivity. The stimulated insulin production was not changed during our study. Parameters of glucose metabolism were similar in patients treated with sirolimus and mycophenolate mofetil.

	RAPA group (n=13)		MMF group (n=13)	
	Examination 1	Examination 2	Examination 1	Examination 2
BMI	22.9±2.3	24.6±2.5 (p=0.05)	23.1±3.9	24.1±3.7 NS
HbA1c (%)	6.4±1.0	5.6±0.49 (p<0.05)	5.8±0.5	5.5±0.49 NS
Fasting glycemia (mmol/L)	5.2±0.72	4.7±0.51 (p<0.05)	5.4±0.82	4.68±0.89 (p=0.01)
K_G (%/min.)	1.03±0.52	1.26±0.39 NS	1.02±0.34	1.55±0.59 (p=0.01)
AUC-IRI (mIU/L/60 min.)	1853±1042	1944±950 NS	2555±1055	2564±1353 NS
AUC-CP (pmol/mL/60 min.)	140.4±65.2	90.7±31 (p<0.01)	151.8±43.1	110.5±29.6 (p<0.01)
HOMA-IR	2.88±1.87	2.3±1.14 NS	4.11±2.76	2.65±2.27 NS

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0239

Which to prefer: Simultaneous islet-kidney or pancreas-kidney transplantation in type 1 diabetes mellitus: A six year single center follow-up

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Background and Aims: Steroid-free immunosuppression has given hope that islet transplantation alone (ITA) is developing into an alternative to pancreas transplantation in patients with type 1 diabetes mellitus. We compared glycemic control and renal function in recipients of simultaneous islet-kidney (SIK) with recipients of pancreas-kidney (SPK) transplants in our hospital.

Materials and Methods: Glycated hemoglobin, need for exogenous insulin and renal function (creatinine/creatinine clearance) were compared in 13 patients with SIK with a mean of 2.2 islet transplantations and 25 patients with SPK transplanted during the same time period (January 2000 – December 2004).

Results: The mean follow-up was 40.5 (12–67) months in both groups. Adverse outcomes: One primary non-function (PNF) of the kidney (retransplanted), one PNF of islets, and 2 incidents of minor liver bleedings in the SIK versus 44% relaparotomies in the SPK group. The mean HbA1c during follow-up in the SIK group as compared to the SPK group was 6.4±0.9 (SD) % vs. 5.9±0.5% (p=0.01) (pretransplant: 8.4±1.8% vs. 8.7±1.9%). 5 out of 7 patients (71%) with ≥2 islet transplantations were initially off insulin (currently 2) as compared to all 25 patients in the SPK group (currently 24). The higher creatinine and lower creatinine clearance in the SIK group (177±130 μmol/l and 45.5±20.9 ml/min/1.73 m^2) as compared to 109±30 μmol/l and 65.0±14.9 ml/min/1.73 m^2 (p<0.01) in the SPK group is most likely due to the 15 years higher donor and 13 years higher recipient age.

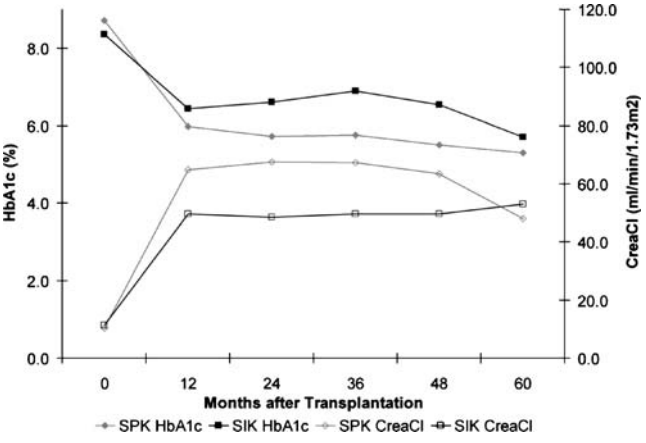

Figure 1. HbA_{1c} and Creatinine Clearance of the SPK and SIK group over 60 months

Conclusion: This series, using about the same number of pancreata for transplantation for SIK or SPK with a mean follow-up of over 3 years indicates that SPK achieves a much higher insulin independence at the cost of more surgical complications, whereas glycemic control is only slightly different in both groups. Residual insulin production combined with optimal insulin therapy seems to be sufficient for maintaining near normal glucose control which is the primary objective of islet transplantation in our institution and not insulin independence alone.

0240

Pancreas transplant alone (PTA) in type 1 diabetic patients (T1DM): a 3-year follow-up

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Background and Aims: Whereas combined pancreas and kidney transplantation is a recommendable procedure in uremic T1DM, the role of PTA in T1DM without advanced kidney disease is still debated. We report our single centre experience on PTA.
Materials and Methods: Between April 2001 and December 2005, 63 T1DM (M/F: 32/31; age: 37±8 yrs; body mass index: 24±3 kg/m^2; duration of diabetes: 23±10 yrs) received a PTA, with portal (49 patients, 77.8%) or systemic (14 patients, 22.2%) endocrine drainage. Indications were hyperlability of diabetes in 9 cases (14.3%), and progressive chronic diabetes complications in the remaining 54 cases (85.7%). Immunosuppression consisted of anti-IL2 receptor antibodies for induction, and MMF, tacrolimus and low dose steroid for maintainance.
Results: Patient survival was 98.2% at 3 years post-transplant, and graft survival (insulin-independence) was 89.8, 87.2 and 79.8% at 1, 2, and 3 years, with graft losses due to immunological reasons. Successful PTA determined sustained normoglycemia (at 1, 2 and 3 years fasting plasma glucose was 86±11, 86±12 and 88±11 mg/dl; A1c was 5.3±0.4, 5.3±0.4 and 5.4±0.4%), with fasting C-peptide levels of 2.8±1.6, 2.7±1.4 and 2.9±1.5 ng/ml. Several cardiovascular risk factors (blood pressure, total and LDL-cholesterol, fibrinogen) improved significantly after PTA, and daily proteinuria decreased from 1.3±2.3 to 0.4±0.8 g ($p<0.01$). No new case of diabetic retinopathy (DR) occurred, whereas 82% of patients with DR showed improvements or stabilization; however, 14% of subjects with advanced DR had deterioration of retinal status. Neuropathy parameters improved significantly in all tested PTA recipients. The observed improvements in PTA were statistically significant when compared with data from non-transplanted, matched T1DM. No cancer disease occurred in PTA recipients.
Conclusion: In conclusion, PTA in T1DM determines prolonged insulin-independence in the majority of patients, with a favourable (although not yet optimal) benefits/risks ratio; most appropriate timing for the procedure and effects on life expectancy remain major open issue.

OP 43

Continuous glucose monitoring

0241

Association between nocturnal hyperglycaemia and severe sleep-disordered breathing in type 2 diabetic patients: a study based on continuous glucose monitoring

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Background and Aims: It has been previously shown that sleep-disordered breathing (SDB) is pathophysiologically related to impaired glucose homeostasis. The aim of this study was to evaluate changes in interstitial glucose levels monitored by continuous glucose monitoring system (CGMS, Medtronic MiniMed) in selected type 2 diabetic patients with or without SDB (verified by polysomnography) and study the possible relationships between nocturnal hyperglycemia and sleep apnea.
Materials and Methods: 30 patients with type 2 diabetes and near-normal blood sugar levels on oral hypoglycemic therapy (22 men, 8 women; mean age±SD: 49,23±4,81 years, HbA1c/HPLC method 6,72±0,73%, duration of diabetes 2,7±1,44 years, BMI 32,46±7,41) were monitored using the CGMS for several days, in conjunction with self-monitoring of blood glucose at least 4 times/day. Patients were divided into two groups according to previous history of SDB. Subjects with severe SDB were referred to sleep laboratory for parallel polysomnographic evaluation of disturbed sleep. We evaluated and compared the CGMS data between both groups. The primary endpoint was the area under the interstitial glucose concentration curve $AUC>_{7,8\ (0–480\ min)}$ for a 8-hour period of night (10PM–6AM). The secondary parameters assessed were: nocturnal and daytime CGMS–glucose values, apnea–hypopnea index (AHI), oxygen saturation parameters, HbA1c. Results were presented as mean±SD. Statistical analyses included correlation, mean absolute difference (MAD) and Student's t-test.
Results: Patients were each monitored with CGMS for an average of 98,67±26,24 h (r=0,86±0,09; MAD=14,37±3,5%). The parallel polysomnographic recording in the SDB-group (n=8; HbA1c 7,36±0,45%; AHI 57,64±9,64/h; oxygen saturation 82,5±5,58%; minimal oxygen saturation 49,13±10,19%) revealed frequent episodes of sleep apnea/hypopnea with severe oxygen desaturation followed by a significant rise in blood glucose of up to 12,3 mmol/l. The nocturnal increment in blood glucose expressed as

$AUC>_{7,8\ (0–480\ min)}$ was significantly lower for non-SDB group (n=22; HbA1c 6,49±0,68%; AHI <10/h) as compared to group of patients with severe SDB (0,2±0,1 mmol/l.night vs. 1,11±0,2 mmol/l.night; p<0,01) and strongly correlated with severe oxygen desaturation (r=0,72, p<0,01). CGMS showed mean nocturnal glucose val ues significantly higher in SDB group (8,24±0,99 mmol/l vs. 6,15±1,16 mmol/l; p<0,001). The peak of nocturnal glucose levels tended to appear within 1 hour post hypoxia. Mean duration of posthypoxic hyperglycemia was 48,2±9,79 min. Morning fasting glucose level was significantly higher in SDB group (8,01±0,19 mmol/l vs. 6,6±0,4 mmol/l; p<0,05). No significant difference in other daytime CGMS-glucose levels was observed between both groups.

Conclusion: The results suggested that sleep apnea may have an immediate elevating effect on nocturnal blood glucose even in patients with near-normal blood sugar level on oral hypoglycemic therapy, but without adequate treatment of severe SDB. These findings indicated that satisfactory treatment of sleep apnea should be considered as an important part of the complex management of diabetic patients with severe SDB in order to prevent postapnoeic nocturnal hyperglycemia and to maintain long-term tight glycemic control.

0242

CGMS reveals critical point in glycaemic excursions requiring therapeutic intervention in people with type 2 diabetics with near-normoglycaemia on long acting sulfonylurea

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Background and Aims: The greatest postprandial glucose excursion in type 2 diabetes occurs after breakfast. The aim of this study was to examine plasma glucose profiles of people with type 2 diabetes with near-normoglycaemia, using continuous glucose monitoring (CGMS, Medtronic MiniMed) to ascertain the frequency of post-breakfast hyperglycaemia and whether this could be modified by carbohydrate intake.

Materials and Methods:30 people with type 2 diabetes on long-acting sulfonylurea monotherapy (14 men, 16 women; mean age±SD: 57.53±6.63 years, HbA1c/HPLC method 6.68±0.76%, BMI 29.13±3.26) were divided into two groups according to amount of carbohydrates eaten in breakfast (≤15 g; n=15 and ≥30 g; n=15). CGMS was applied for several days during normal activity along with self-monitoring of blood glucose at least 4 times/day. Patients recorded: times of meals, exercise, and hypoglycaemic drug administration. The primary endpoint was the area under the interstitial glucose concentration curve $AUC>_{7,8\ (0–120\ min)}$ for breakfast. The secondary parameters assessed were mean CGMS-glucose values for breakfast, lunch and dinner. Results were presented as mean±SD. Statistical analyses included correlation, mean absolute difference (MAD) and Student's t-test.

Results: All subjects were each monitored with CGMS for an average of 113.63±22.85 h (r=0.82±0.07; MAD=12.82± 2.5%). $AUC>_{7,8\ (0–120\ min)}$ in group with ≤15 g carbohydrates in breakfast was significantly lower than in the group of ≥30 g carbohydrates (0.4±0.3 mmol/l.h vs. 4.34±1.88 mmol/l.h; p<0,001). $AUC>_{7,8\ (0–120\ min)}$ for breakfast strongly correlated with the amount of breakfast carbohydrates (r=0.82). The mean CGMS-glucose values were: fasting, 7.3±2.2 mmol/l; 1.5 h post-breakfast, 11.6±2.27 mmol/l; pre-lunch, 6.3±2.59 mmol/l; 1.5 h post-lunch, 8.03±2.89 mmol/l; pre-dinner, 6.4±2.1 mmol/l; 1.5 h post-dinner, 8.1±2.3 mmol/l. Despite the comparable carbohydrates intake during the day, the highest peak of post-meal hyperglycemia occurred after breakfast.

Conclusion: The CGMS results demonstrated that post-breakfast hyperglycemia appears to be most important time for glycaemic control even in patients with near-normoglycaemia. From the results of this study, 15 G of carbohydrate appears to be the maximum amount in breakfast to prevent an excessive postprandial hyperglycaemic excursions in most people with 2 diabetes.

0243

Glycaemic control improvement with the weekly use of the Guardian^{RT} together with a wireless assistant for asynchronic remote advice in pump treated type 1 diabetic patients

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Background and Aims: The s.c. continuous glucose monitoring system Guardian^{RT} allows the patient to have real time access to the interstitial glucose data. The PARIS_Diabtel system provides physicians and patients of 24 hours telemonitoring and telecare services based on web

applications and a wireless personal assistant (PA). We sought to evaluate, in terms of metabolic control, the use of the Guardian^{RT} in addition to the PARIS_Diabtel system in a randomized cross-over clinical.

Materials and Methods: Ten type 1 diabetic patients (5 women, age 40.6 (21–62), diabetes duration 14.7 (3–52) years) treated with insulin pumps for more than a year, were included. Along the study, patients used the PARIS_Diabtel system for glucose and insulin data transmission to the diabetes center, analysis by the physician who advised modifications of the basal rate and boli. During the 4-weeks intervention phase, patients used 3 days a week the Guardian^{RT}. At the end of the control phase a 3-day glucose monitoring (MiniMed CGMS).

Results: After the intervention phase, HbA1c decreased significantly (8.1±1.1 vs. 7.3±0.8; p=0.01) while no significant changes were observed during the control phase. Data regarding the use of the system (mean number of daily glucose values and boli sent by patients, PA functionalities: data visualization, data download from the insulin pump and synchronization with the Telemedicine Server as well data of the therapy changes advised and performed by patients will be presented. We are now analysing the results of the s.c. glucose monitoring performed in both study phases using the poincare plot described by Kovatchev B. et al.

Conclusion: The Guardian^{RT} together with the PARIS_Diabtel system, a telemedicine system that includes a wireless personal assistant for remote treatment advising, improves glycemic control in pump treated type 1 diabetic patients

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0244

Improvement in glucose excursions using a seven-day continuous glucose sensor: managing the extremes

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Background and Aims: Hypoglycaemia and wide glucose excursions continue to be major obstacles to achieving target HbA1c values and the associated reductions in long-term complications (and economic costs) in people with insulin-treated diabetes. In this study we evaluated the accuracy, safety, and clinical effectiveness of a continuous glucose-sensing device.

Materials and Methods: Baseline HbA1c was measured within 30 days of enrollment of 86 patients with insulin-requiring diabetes who participated in this trial. Patients inserted and wore a transcutaneous glucose sensor (STS System, DexCom, Inc.) for 21 days in three 7-day periods during normal activities. Sensors were BLINDED during period 1. During periods 2 and 3 (DISPLAY), glucose values, trend graphs, and high/low alerts and alarms were provided.

Results: Of 6357 paired sensor-SMBG points prospectively analyzed, mean absolute relative difference was 15.7%. Patients with HbA1c > 9% spent 94.6% (median) more time in euglycaemia (81-140 mg/dL) during the unblinded period without an increase in hypoglycaemia (Table 1A). Patients with HbA1c ≤ 7% maintained euglycaemia as in the blinded state but spent 46.4% (median) less time below 55 mg/dL during DISPLAY use (Table 1B).

Conclusion: We conclude that 7-day use of the STS System was safe, accurate, and stable. Moreover, patients with poorly controlled diabetes can use the added information from a continuous sensor to significantly improve glycaemic profiles without increased risk of hypoglycaemia. The well-controlled patient can also maintain their glucose control while using a continuous sensor, but have a reduced incidence of hypoglycaemia.

Patients with A1c<7%

Glucose Range (mg/dL)	Median Percent Change in Time Spent (BLINDED vs. DISPLAY)	p-value
<55	**−46.4%**	**0.0316**
55–80	−14.5%	0.1046
81–140	18.8%	0.1705
141–240	−8.5%	0.6099
241–400	−14.2%	0.7243

Patients with A1c >9%

Glucose Range (mg/dL)	Median Percent Change in Time Spent (BLINDED vs DISPLAY)	p-value
<55	−31.1%	0.4238
55–80	27.4%	0.4697
81–140	**94.6%**	**0.0068**
141–240	0.8%	0.4697
241–400	−36.4%	0.6772

Supported by DexCom, Inc.

OP 44

Transcriptional regulation of beta cell function

0245

Construction of a pancreatic beta-cell specific gene atlas

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Background: Understanding and exploring the basal gene expression in a given tissue provides new insights in the functioning of genes under normal and disease conditions. Genes that are specifically or highly expressed in pancreatic islets are candidates for biomarkers which can potentially serve as an indicator of physiological or disease processes in diabetes.
Material and Methods: Massively Parallel Signature Sequencing (MPSS) is a sequencing-based technology to detect and quantify the expression of almost all known genes by counting the number of short sequence signatures. To explore the transcriptome of human pancreatic islets and prepare a comprehensive inventory of insulin-producing cell gene expression, we performed MPSS analysis of two human untreated pancreatic islet samples. A total of 1,051,000 and 1,313,239 signatures were sequenced for samples 1 and 2, respectively. The abundance of each signature was represented as transcripts per million (tpm). With the aim to determine genes that are restricted to β cells, gene expression profiles of FACS-purified primary β cells and INS cell lines were detected by Affymetrix Rat230 2 chip. The secreted and transmembrane proteins were predicted using SecretomeP 3.0 and TMHMM 2.0, respectively.
Results: After filtering the bad quality signatures with hits to multiple Unigene clusters and applying a stringent cut-off threshold of 10 tpm, we identified 3869 shared unique genes in both samples and 6939 genes in one or both human pancreatic islet samples. MPSS method is particularly sensitive in determining low abundance transcripts that microarray platforms fail to detect. Comparison of MPPS results with published microarray datasets performed with untreated human pancreatic islets identified 1036 genes that could be detected by MPSS analysis alone. The majority (736) of these genes were low abundant transcripts with counts lower than 30 tpm. We subsequently compared islet MPSS results to a published MPSS dataset sampling a collection of 31 normal human tissues. This analysis revealed 848 unique genes that are either specific to or highly enriched in human islets. This list contains 114 putative transmembrane proteins of which 44 are highly expressed in primary β cells. Similarly, the list of islet-enriched genes contains 228 classic (signal peptide-triggered) or non-classical (lacking a signal peptide) secreted proteins that are also expressed strongly in primary β cells. In order to complement our MPSS data, we collected and analyzed almost all published microarray gene expression studies from endocrine and exocrine pancreas from different species. We have constructed a database of pancreas gene expression atlas and will make it publicly available via, T1DBase (http://t1dbase.org), a T1DM-specific bioinformatics resource.
Conclusion: Our pancreas gene atlas provides interesting insights on the gene expression landscape of pancreatic β cells, and is a valuable source for detailed information on the gene expression distribution in beta cells and pancreatic islets. Moreover, the list of pancreatic islet enriched genes contains proteins that are secreted or reside in plasma membrane and have the potential to serve as β cell specific markers.
Supported by the Juvenile Diabetes Research Foundation

0246

Global analysis of the transcriptional role of HNF-1α in pancreatic islets

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Background and Aims: The most common form of monogenic diabetes (MODY 3) is caused by mutations in the gene encoding HNF-1α (also known as TCF1). Lack of HNF-1α has a dramatic effect on beta-cell function, which indicates that HNF-1α targets play key functional roles in the beta cell. Despite knowledge of several genes that are regulated by HNF-1α in mouse islets, a complete picture of the role of HNF-1α in beta cells remains elusive. We have combined genome-wide expression profiling and transcription factor binding to address the role of HNF-1α as a regulator of a beta-cell transcriptional programme.
Materials and Methods: Expression profiles of cultured islets from control and *Hnf1*α−/− mice were analyzed with Affymetrix chips. Statistical analysis of the data was carried out with SAM, and genes exhibiting differential regulation at a 5% false discovery rate were selected. HNF-1α binding to targets genes was analyzed by chromatin immunoprecipitation combined with promoter microarrays from the Beta Cell Biology Consortium and custom arrays produced

by Agilent. High affinity, evolutionary conserved HNF1 consensus *cis* elements were identified after aligning the mouse, human, dog, and chicken genomic sequences.
Results: HNF-1α plays an unexpectedly wide-ranging role in regulating gene expression in the pancreatic islets, with significantly increased or reduced expression of over 15% of active genes. To distinguish between direct and indirect effects of HNF-1α, we carried out a computational analysis of evolutionary conserved HNF1 sites in the mouse genome and chromatin immunoprecipitation/microarray analysis of HNF-1α-bound chromatin. This revealed that HNF-1α primarily acts as an essential activator in a substantial number of genes, but is unlikely to carry out direct repressor functions in islets. It also showed that HNF-1α directly regulates a disporportionate number of genes regulating specific biological processes, most notably genes encoding secreted regulatory proteases, transcriptional regulators, and regulators of amino acid and glucose metabolism. Interestingly, this study also uncovered an altered expression of genes involved in important signaling events for the beta cell. Genes that exhibit full dependence on HNF-1α were more likely to be direct targets. In contrast, indirect targets are functionally pleiotropic and exhibit only moderate dependence on HNF-1α.
Conclusion: These findings provide a framework to understand the structure of a key beta-cell genetic programme, and suggest unanticipated biological insights into the role of the MODY3 gene in the beta cell.
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0247

SREBP-1c antagonises PDX1 functions through the direct interaction and suppresses PDX1 expression
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Background and Aims: Overexpression of SREBP-1c in β-cells caused impaired glucose-stimulated insulin secretion associated with diminished PDX1 expression *in vitro* and *in vivo*, suggesting that a potential role of SREBP-1c in the lipotoxicity could be mediated through direct suppression of PDX1. The aim of this study is to clarify the molecular mechanism of PDX1 repression by SREBP-1c.
Materials and Methods: Promoter analysis for a mouse PDX1 gene (2.7 kb) was performed in β-cells (HIT) and non-β-cells (HepG2) with a luciferase reporter gene system. The direct interaction of PDX1 and SREBP-1c was estimated by GST pull-down and EMSA assays. Gene expression was investigated by Northern blot analysis in β-cells (INS1) after adenovirus-mediated overexpression of SREBP-1c and GFP as a control.
Results: In luciferase assays, the promoter activity of the mouse PDX1 gene was not affected by co-expression of nuclear SREBP-1c in HepG2 cells, but strongly suppressed in HIT cells. PDX1 itself activated the promoter, but SREBP-1c completely antagonized PDX1 feedforward-loop activation in both cells. To investigate the inhibitory mechanism by SREBP-1c, a series of deleted PDX1 promoter activities by SREBP-1c were examined. The *cis* element for SREBP-1c was not identified on the promoter. Next, protein–protein interaction was examined by GST pull-down assay. SREBP-1c directly interacted with PDX1. The PDX1 *cis* element was previously reported in the distal enhancer region (Area I). EMSA with radiolabeled probe of Area I demonstrated that SREBP-1c dose-dependently reduced PDX-1 binding ability to the Area I, whereas SREBP-1c did not bind to this region, excluding the possibility that SREBP-1c might suppress PDX1 promoter through their direct binding. Adenovirus mediated SREBP-1c overexpression in INS1 cells suppressed both PDX1 and insulin mRNA levels.
Conclusion: These results demonstrate that SREBP-1c antagonizes PDX1 functions through protein–protein interaction and reduced PDX1 expression potentially leading to defects in insulin secretion and β-cell mass as a potential role of SREBP-1c in β-cell lipotoxicity.
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0248

PTB1 binds to the 5′-UTR of mRNAs encoding proteins of the insulin secretory granules
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Background and Aims: The biosynthesis of insulin secretory granules (SG) is regulated by glucose. Previously we have shown that in rat islets and cultured insulinoma INS-1 cells glucose and glucagon-like peptide 1 (GLP-1)

rapidly increase the expression of various SG proteins through the activation of a posttranscriptional pathway involving polypyrimidine tract-binding protein (PTB1). Upon its glucose-stimulated nucleocytoplasmic translocation, PTB1 binds to the 3′-untranslated regions (UTR) of SG mRNAs, including ICA512, PC1/3 and PC2, thereby leading to their stabilization and increased translation. Especially in view of its role in translation, we investigated here whether PTB1 can also directly bind to the 5′-UTR of ICA512, PC1/3 and PC2 mRNAs, and whether such binding is regulated by glucose.

Material and Methods: The 5′-UTR of rat ICA512 was extended beyond its previously known boundaries by 5′-RACE. The 5′-UTRs of rat ICA512, PC1/3 and PC2 were biotinylated *in vitro* and incubated with cytosolic extracts from INS-1 cells non-stimulated (0 mM glucose) or stimulated (25 mM glucose) for 2 hrs. After RNA pull-down with streptavidin-magnetobeads, binding of PTB1 was analysed by immunoblot. The same 5′-UTRs have been cloned upstream of the firefly luciferase cDNA in the pGL3 vector (Promega) and co-transfected with renilla luciferase in INS-1 cells to assess the ability of these 5′-UTRs to enhance luciferase activity in response to glucose.

Results: Bioinformatic analyses indicated the presence of a canonic consensus sequence for PTB1 binding (–CYYYY CYYYYYG–) in the 5′-UTRs of PC1/3 and PC2, but not in the 5′-UTR of rat ICA512. The latter only includes a short pyrimidine-rich sequence. Nevertheless we found that PTB1 binds also to the 5′-UTR of ICA512. The recovery of PTB1 was ~3 fold higher when each of the three biotinylated 5′-UTRs was incubated with cytosolic extracts from glucose stimulated INS-1 cells. Inclusion of the PC2 5′-UTR enhanced firefly luciferase activity by ~15 fold in resting INS-1 cells and ~56 fold upon glucose stimulation compared to pGL3 alone. These increments were abolished by mutation of the PTB1 binding site in the PC2 5′-UTR. The impact of the ICA512 and PC1 5′-UTRs on luciferase expression is currently under investigation.

Conclusions: Our studies provide evidence that in response to glucose stimulation PTB1 promotes not only the stability but also the translation of several mRNAs encoding SG proteins by binding to their 5′-UTRs. These findings are consistent with the ability of PTB1 to enhance the translation of viral mRNAs by binding to motifs in their 5′-UTRs.

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OP 45

Retinopathy – clinical

0249

Effect of ruboxistaurin on diabetic macular edema and visual loss: integrated analysis of the PKC-DRS and PKC-DRS2

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Background and Aims: Persons with advanced non-proliferative diabetic retinopathy (NPDR) are at increased risk of vision loss. The PKC-DRS, a multidose trial in 252 patients, evaluated whether ruboxistaurin (RBX), an oral protein kinase C β inhibitor, slowed progression of NPDR to proliferative diabetic retinopathy. No effect was seen for this outcome, but RBX did reduce the 3-year occurrence of sustained moderate visual loss (SMVL; a ≥15-letter loss on the ETDRS chart sustained for the last 6 months of study participation). The PKC-DRS2 evaluated these outcomes in 685 patients at the dose with greatest effect in the PKC-DRS. We report data for placebo (n=401) and 32 mg/d RBX (n=412) groups combined from both trials.

Material and Methods: Both the PKC-DRS and PKC-DRS2 were 3-year, multi-center, randomized, placebo-controlled, double-masked, phase 3 trials. Patients had a best-corrected ETDRS visual acuity (VA) ≥45 letters (approximately 20/125 Snellen=6/40=0.16, or better), moderately severe to very severe NPDR (ETDRS retinopathy level ≥47A/47B and ≤53E), and no prior panretinal photocoagulation in at least one eye. There were no restrictions on the presence of diabetic macular edema (DME) or prior focal/grid photocoagulation at baseline.

Results: For the combined population, mean HbA1c was 8.2% and mean systolic and diastolic blood pressures were 138 and 78 mm Hg at baseline. SMVL occurred in 10.2% of placebo vs. 6.1% of RBX patients (41% risk reduction, P=0.011). A significant between-group difference in mean VA change from baseline was observed starting at

12 months of therapy, and continued through the duration of the studies. A ≥15-letter gain of VA occurred in 2.4% of placebo vs. 4.7% of RBX eyes (P=0.021), and a ≥15-letter loss of VA occurred in 11.4% of placebo vs. 7.4% of RBX eyes (P=0.012). Of placebo and RBX eyes with clinically significant macular edema (CSME) >100 μm from the center of the macula at baseline, 66.9% vs. 50.0%, respectively, progressed to having CSME ≤100 μm from the center of the macula (P=0.002).

Conclusion: Combined analyses of these two similarly designed trials demonstrated that, compared with placebo, RBX reduced the occurrence of vision loss, reduced the progression of DME to the center of the macula, and increased the chance of VA improvement in patients with moderately severe to very severe NPDR.

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0250

Proteomic analysis of human vitreous fluid by DIGE: a new strategy for identifying potential candidates in the pathogenesis of proliferative diabetic retinopathy

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Background and Aims: Vitreous fluid analysis has been used as a surrogate of the metabolic events that are taking place in the retina *in vivo*. However, the volume of vitreous fluid obtained after vitrectomy is about 1 ml and, therefore, only a few peptides can be analyzed simultaneously. The recent development of proteome analysis has made it feasible to compare protein profiles in body fluids with only a small sample and, therefore it could be a useful tool in exploring new candidates potentially involved in the pathogenesis of diabetic retinopathy. Fluorescence-based Difference Gel Electrophoresis (DIGE), a method that permit us to perform an accurate quantitative comparison, has not been previously used in the proteomic analysis of the vitreous fluid in the setting of diabetic eye disease. The aim of the study was to compare for the first time the protein profiles of human vitreous from diabetic patients with proliferative diabetic retinopathy (PDR) with those obtained from non-diabetic patients with idiopathic macular holes (MH) by using DIGE strategy.

Materials and Methods: Vitreous humors from type 1 diabetic patients with PDR (n=8) and from non-diabetic patients with MH (n=10) closely matched by age were included. Vitreous hemorrhage was ruled out by measuring intravitreal hemoglobin. DIGE strategy was used to carry out the comparative proteomic analysis. In order to improve the coverage of the proteins present in the vitreous we have used an affinity chromatographic separation to deplete the highest abundant proteins (ie. IgG and albumin) before their electrophoretic separation. Differentially expressed proteins (abundance ratio >1.4 fold; $p<0.05$) were identified by mass spectrometric analysis.

Results: 8 proteins were highly expressed in PDR patients in comparison with non diabetic subjects (Zinc-α-2-glycoprotein -ZAG-, Apo A1, Apo H, Fibrinogen A, and the following complement factors: C3, C4b, C9 and Factor B). By contrast, we found 3 proteins underexpressed in PDR subjects (Pigment epithelial derived factor -PEDF-, Interstitial retinol-binding protein -IRBP-, and Inter-alpha tripsin inhibitor heavy chain -ITIH2-). There was no overlap in the vitreous levels of the above-mentioned proteins between PDR patients and non-diabetic controls. The different expression of ZAG, C3, Factor B, PEDF and IRBP was further confirmed by Western blot.

Conclusion: Proteomic analysis by DIGE, which permits an accurate quantitative comparison, was useful in identifying new potential candidates involved in the pathogenesis of PDR.

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0251

Deficit of somatostatin expression in diabetic human retina: an early event in the pathogenesis of diabetic retinopathy

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Background and Aims: Somatostatin (SST) and its receptors are found in the neuroretina of various species, including humans, and there is growing experimental evidence that in the retina SST acts as both a neuromodulator and antiangiogenic factor. In fact, we have previously shown that SST exists in abundant quantities in the human vitreous fluid, thus supporting the concept that it is essential in retinal homeostais. In addition, lower intravitreous SST levels were found in patients with proliferative diabetic retinopathy than in nondiabetic subjects.

Although vitreous fluid analysis can be considered as a surrogate of the events that are taking place in the retina, studies addressed to confirm the deficit of SST production by the retina of diabetic patients have not been previously reported. The aim of the present study is to compare mRNA SST in the retina from diabetic and nondiabetic donors. In addition, the differential SST expression between neuroretina and retinal pigment epithelium (RPE) was also explored for the first time.

Materials and Methods: Human postmortem eyes (n=16) were obtained from 4 diabetic donors without retinopathy and 4 non diabetic donors age-matched. The time elapsed from death to eye enucleation was 3.8±1.4 h. SST mRNA expression was determined by semi-quantitative RT-PCR. To evaluate the quantitative expression of the SST mRNA a Quantitative RT-PCR was performed using specific primers (TaqMan® pre-made gene expression assay Hs00174949-m1, Applied Biosystems) in an ABI Prism 7000 Sequence Detection System (PE Applied Biosystems).

Results: A significant higher expression of SST mRNA in the RPE than in the neuroretina in retinas from nondiabetic donors was detected (0.98±0.02 *vs.* 0.38±0.24; p<0.001). In retinas from diabetic donors, SST mRNA expression was also higher in the RPE than in the neuroretina (0.44±0.30 *vs*. 0.11±0.09; p=0.018). Finally, SST mRNA levels were lower in both RPE and neuroretina from diabetic donors in comparison with non-diabetic donors (0.44±0.30 *vs.* 0.98±0.02; p=0.001 and 0.11±0.09 *vs.* 0.38±0.24; p=0.018, respectively).

Conclusion: 1) Apart from neuroretina, RPE is a significant contributor to intraocular SST production. 2) A significant deficit of SST mRNA expression were found in both neuroretina and RPE from diabetic donors. 3) Since diabetic donors were free of clinically detectable microvascular changes, SST deficit can be considered as an early event in the pathogenesis of diabetic retinopathy. Finally, our results suggest that intravitreous SST delivery would be considered a more rational approach than systemic administration to prevent or arrest diabetic retinopathy.

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0252

Erythropoietin is expressed in human retina and it is highly elevated in the vitreous fluid of patients with diabetic macular edema

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Background and Aims: Erythropoietin (Epo) has been recently found increased in the vitreous fluid from ischemic retinal diseases such as proliferative diabetic retinopathy (PDR). The aims of the present study were: 1) To measure Epo levels in the vitreous fluid from patients with diabetic macular edema (DME), a condition in which the ischemia is not a predominant event. 2) To compare Epo mRNA expression between human retinas from non-diabetic and diabetic donors without retinopathy.

Materials and Methods: Vitreous from 12 type 2 diabetic patients with DME without significant retinal ischemia and 12 PDR patients were prospectively analysed. Vitreous from 10 non-diabetic patients with macular holes served as the control group. Epo was assessed by RIA (mU/ml). Epo m-RNA expression was measured by quantitative real-time RT-PCR analysis in the retina from 8 non-diabetic and 8 age-matched diabetic donors without diabetic retinopathy.

Results: Intravitreal Epo concentration was higher in both PDR and DME patients than in non-diabetic controls (PDR vs. controls: 302 [117–1850] *vs.* 30 [10–75], p<0.01; DME vs. controls: 430 [41–3000] *vs*. 30 [10–75], p<0.01). Higher intravitreous Epo concentration was detected in subjects with DME in comparison with PDR patients, but the difference was not statistically significant either in absolute terms or after adjusting for intravitreal proteins (430 mU/ml [41–3000] *vs*. 302 mU/ml [117–850]; p=0.21 and 105 mU/mg [13–967] *vs*. 65 mU/mg [32–1027]; p=0.53, respectively). Epo mRNA expression was detected in the human retina and it was higher in the retina from diabetic than from non-diabetic donors. In addition, higher expression of Epo mRNA was detected in retinal pigment epithelium than in the neuroretina in both, diabetic donors and non-diabetic donors.

Conclusion: Intravitreous Epo concentration is strikingly elevated in patients with DME without significant retinal ischemia. In addition, Epo is expressed in the human retina and it is upregulated in diabetic donors even without retinopathy. These findings strongly suggest that other factors apart from ischemia are involved in the over-expression of Epo in diabetic retinopathy.

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OP 46

Modulation of inflammatory markers in diabetes

0253

Treatment with interleukin-1 receptor antagonist improves glycaemic control and β-cell function in patients with type 2 diabetes mellitus

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Background and Aims: High concentrations of glucose induce β-cell production of interleukin-1(IL-1)β leading to impaired β-cell function and apoptosis in isolated human islets. Islet production of natural IL-1 receptor antagonist (IRAP) is reduced in Type 2 diabetes (T2D). Therefore, the balance of islet derived IL-1 and IRAP may play a crucial role in progressive β-cell failure in T2D. *Aim*: To investigate if IRAP improved glycemia and β-cell function in patients with T2D.
Materials and Methods: In an investigator-initiated, randomised, placebo-controlled, double-masked two-centre clinical trial, 70 out-patients with T2D (age 60.1 yrs, 27% female, diabetes duration 11 yrs, baseline BMI 31.4, HbA1c 8.4%, basal C-peptide 1061 pM (ambient pl-glucose 10.3 mM), 2% on diet, 47% on oral agents (OA), 51% on OA+insulin or insulin alone) were randomized (sequence and code concealment by County Pharmacy Zürich) to treatment with 100 mg sc of rhIRAP (Kineret, Amgen, prepared and labelled by County Pharmacy Zürich) once daily or placebo (P) for 13 w and followed up for 39 w. Diet and anti-diabetic therapy were unchanged during IRAP treatment. At baseline and 13 w an OGTT followed by 0.3 g/kg glucose, 0.5 mg glucagon and 5 g arginine iv (IV) and in 36 random patients hyperinsulinemic-euglycemic clamps were performed after overnight fast, no exercise and no anti-diabetic medication. ΔHbA1c was **1° end-point** and Δ-β-cell function and -insulin sensitivity 2° end-points between baseline and 13 w. HbA1c was measured (DCCT/IFCC standards) with exchange between the two centers, C-peptide and insulin by ELISA at SDC and data analysed as per-protocol. 42 patients were required to obtain 80% power to detect a change in Hba1c of 0,5% (one-sided significance of α=0.05).
Results: HbA1c (but not Hb) and 120 min pl-glucose in OGTT decreased in IRAP- and increased in placebo-treated patients (−0.46±0.2 vs 0.08±0.12%, p=0.011; −0.6±0.5 vs 1.0±0.8 mM, p=0.04, respectively). ΔC-peptide (total AUC (AUCt) during OGTT) improved by IRAP- but deteriorated by placebo-treatment (10.5±6.8 vs −8.2±7.8 nMxmin, p=0.038), as did the AUCt during OGTT+IV test (p=0.041). IV ΔAUCt-insulin was also improved in IRAP treated patients (1.2±0.5 vs −0.2±0.5 nMxmin, p=0.031). IRAP did not alter BMI or insulin sensitivity, but reduced C-reactive peptide (−4.4±1.6 vs 0.3±0.3 mg/l, p<0.001), and was well-tolerated, apart from self-limiting local injection reactions in 10 patients.
Conclusion: Blocking IL-1 action for 13 weeks improves glycemic control and β-cell function, but does not alter BMI or insulin sensitivity in poorly controlled T2D patients. This study provides proof-of-principle that IL-1 is an important mediator of impaired glycemia in T2D by affecting β-cell function.

0254

Effect of adjunctive pramlintide treatment on hsCRP and adiponectin levels in intensively treated patients with type 1 diabetes

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Background and Aims: Elevated plasma concentrations of high-sensitivity C-reactive protein (hsCRP) and decreased concentrations of adiponectin (ADPN) have been related to risk of cardiovascular disease (CVD) in diabetes. Higher concentrations of hsCRP were observed in intensively treated patients who gained weight in the Diabetes Control and Complications Trial. Low plasma adiponectin concentrations were associated with progression of atherosclerosis in the Coronary Artery Calcification in Type 1 Diabetes study. The aim of the present study was to assess the effects of pramlintide therapy on these markers in intensively treated patients with type 1 diabetes.
Materials and Methods: We performed a post-hoc analysis of a randomized, double-blind study in 187 subjects with type 1 diabetes on intensive insulin therapy. Subjects were treated with either adjunctive pramlintide (30 or 60 μg with major meals, n=84) or placebo (n=103), with both groups targeting similar glycemic goals. Plasma samples were assayed for both hsCRP and ADPN at baseline and Week 25. Baseline characteristics (age 42±14 vs. 42±12 y, disease duration 20±12 vs. 21±12 y, body weight 81.0±17.8 vs. 80.6±16.8 kg, mean±SD) for pramlintide vs. placebo subjects were well balanced, as were metabolic measures (A1C 8.0±0.8 vs. 8.1±0.9%, hsCRP 2.0±1.9 vs. 1.7±1.7 mg/L, ADPN 12.9±7.6 vs. 14.4±9.5 μg/mL).

Results: At 25 weeks, pramlintide improved postprandial glucose control (152±4 vs. placebo 174±4 mg/dL, p<0.001, mean±SE), reduced body weight (−0.93±0.4 vs. placebo +1.38±0.3 kg, p<0.001), decreased mealtime insulin dose (−22±5 vs. placebo+4±4%, p<0.001), and resulted in similar reductions in A1C (−0.4±0.1 vs. placebo −0.6±0.1%) compared with placebo. Placebo subjects had a significant increase in hsCRP compared with pramlintide subjects (+0.66±0.2 vs. −0.06±0.2 mg/L, p<0.01) while ADPN was significantly increased in pramlintide compared with placebo subjects (+0.69±0.5 vs. −0.43±0.3 μg/mL, p<0.05) at Week 25. After 25 weeks of therapy, a significantly larger proportion of placebo subjects shifted to hsCRP levels indicative of higher relative risk (hsCRP>3.0 mg/L) compared with pramlintide subjects after 25 weeks of therapy (19 vs. 5%, p<0.01). Multiple regression analysis, adjusting for baseline hsCRP, treatment, and changes from baseline in A1C and body weight revealed that each of these factors was significantly associated with Week 25 hsCRP. Similar modeling indicated a significant relationship between change in body weight and Week 25 ADPN.

Conclusion: Adjunctive pramlintide in patients with type 1 diabetes intensively treated with insulin therapy lead to an increased concentration of ADPN and an attenuated increase in hsCRP when compared with patients treated with insulin alone, even at similar levels of glycemia. Longer-term studies are warranted to further investigate the clinical relevance of these findings to cardiovascular risk in type 1 diabetes.

0255

Metabolic and anti-inflammatory effects of pioglitazone (PIO) lead to histological improvement in patients with non-alcoholic steatohepatitis (NASH)

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Background and Aims: NASH is a chronic liver condition associated with steatosis, necroinflammation (+/−fibrosis), insulin resistance and glucose intolerance. We have shown that pioglitazone treatment in NASH has beneficial effects on liver histology, although the contributions of improved glucose metabolism and reduction in inflammation remain unclear.

Materials and Methods: We studied 48 IGT/T2DM biopsy-proven patients with NASH (age: 51±2, BMI: 33±1 kg/m^2, FPG: 116±6 mg/dl) and 12 normal controls (CON) matched for age, gender and ethnicity. Patients received a hypocaloric diet and were randomized (double-blind) to PIO (45 mg/d) or placebo (Pbo) for 6 months. Before and after treatment patients had a liver biopsy and a double-tracer OGTT (3-^{3}H glucose infusion/^{14}C in oral glucose load) to assess glucose tolerance/clearance and hepatic/peripheral glucose metabolism. In addition to metabolic parameters, we measured in plasma the following inflammatory markers: hsCRP, interleukin-6 (IL-6), tumor necrosis factor (TNF)-α, tumor growth factor (TGF)-β and vascular- and intracellular adhesion molecules (VCAM/ICAM).

Results: Pbo reduced only AST/ALT (p<0.02) and hepatic inflammation (p<0.03). Compared to CON, pts with NASH had hepatic insulin resistance (fasting hepatic insulin sensitivity index [HISI]=100/[EGP x FPI]; 5.0±1.4 vs. 19.3±2.2) and reduced glucose clearance during the OGTT (2.17±0.2 vs. 3.15±0.3 mg/min, both p<0.01). PIO lowered HbA_{1c} (−0.8%) and ALT/AST to normal, improved HISI by ~60% and increased adiponectin ~2.5 fold (all p<0.0001; p<0.01 vs. Pbo). Steatosis and necroinflammation decreased by ~50% (both p<0.0001, p<0.01 vs. Pbo). PIO significantly increased glucose clearance (2.2±0.2 vs. 2.5±0.2 mg/min, p<0.01; p<0.0001 vs. Pbo). We calculated an index of peripheral insulin sensitivity from the OGTT (Rd/I), by dividing glucose disposal rate (largely muscle) by the plasma insulin. PIO enhanced Rd/I (6.8±0.7 vs. 10.0±0.9 mg/kg min÷μU/ml, p<0.01) but was unchanged by Pbo. Compared to CON, hsCRP was ~3-fold higher in NASH patients, while IL-6, TNF-α, VCAM and ICAM were all 40–50% higher (all p<0.02–0.001 vs. CON). PIO significantly reduced hsCRP (6.1±1.0 to 3.1±0.8 mg/L, p<0.04), TNF-α (2.2±0.1 vs. 2.0±0.1 pg/ml, p<0.01), TGF-β (31±2 vs. 26±2 ng/ml, p<0.05), VCAM (523±32 vs. 480±32 ng/ml, p<0.01) and ICAM (315±17 vs. 268±10 ng/ml, p=0.001). In the PIO arm, steatosis inversely correlated with both basal (r=−0.41) and OGTT (r=−0.51) improvement in Rd/I (both p<0.01).

Conclusion: PIO improves both liver and muscle insulin sensitivity in patients with NASH. These metabolic effects of PIO may be important both for the histological response seen in patients with NASH as well as for the reduction of the pro-inflammatory state associated with this condition.

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0256

Metabolic and histologic improvement by pioglitazone treatment in NASH increases plasma adiponectin levels and reduces visceral and liver fat in patients with NASH

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Background and Aims: We have previously shown that pioglitazone (PIO) treatment in non-alcoholic steatohepatitis (NASH) improves glucose/lipid metabolism, insulin sensitivity, steatosis and leads to marked histologic improvement. However, the relationship between these beneficial metabolic/histologic effects and changes in visceral/liver fat content have not been studied before.
Materials and Methods: We present data on 34 IGT/T2DM biopsy-proven pts with NASH (age: 51±2, BMI: 32±1 kg/m^2, FPG: 118±5 mg/dl) and 8 normal controls (CON) matched for age, gender and ethnicity. Pts received a hypocaloric diet and were randomized (double-blind) to PIO (45 mg/d) or placebo (Pbo) for 6 months. Before and after treatment pts underwent the following studies: 1) liver biopsy; 2) double-tracer 75 gram OGTT (3-^{3}H glucose infusion/^{14}C in oral glucose load) to assess glucose tolerance/clearance and hepatic/peripheral glucose metabolism; 3) measurements of liver fat content by magnetic resonance spectroscopy (MRS) and of visceral (VF)/subcutaneous (SC) fat by magnetic resonance imaging (MRI).
Results: Patients with NASH had increased liver fat by MRS (21±2% vs. 10±2%, p<0.04) and a ~2.5-fold increase in VF (p=0.01) compared to CON. Treatment with PIO for 6 months led to a significant improvement in basal (fasting) hepatic insulin sensitivity, glucose tolerance and glucose clearance during the OGTT, and marked histologic improvement (all p<0.01–0.001 vs. Pbo). Plasma FFA levels decreased by ~30% with PIO while plasma adiponectin levels increased ~3-fold (p<0.001 vs. Pbo) in association with a ~50% reduction of liver fat and ~30% decrease in VF (both p<0.01 vs. Pbo, which was unchanged). In the PIO group, reduction in both liver fat content by MRS and VF were associated with an improvement in glucose clearance (r=0.44 and r=0.55, respectively, both p<0.01). There was a strong correlation between the increase in plasma adiponectin concentration and the decrease in liver fat by MRS (r=−0.57, p<0.001), VF (r=−0.46, p<0.01), as well as enhanced hepatic insulin sensitivity (r=0.37, p<0.01), glucose clearance (r=0.44, p<0.004) and decreased mean glycemia (r=−0.34, p<0.03) during the OGTT. The decrease in liver fat by MRS and VF with PIO occurred despite a mild increase in body weight (PIO=+2.7 vs. Pbo –0.4 kg, p<0.02). The improvement in the total histologic score of steatosis, necroinflammation and fibrosis was directly associated with decrease in visceral fat (r=0.48, p=0.01) and inversely with an increase in plasma adiponectin concentration (r=−0.43, p<0.006).
Conclusion: In pts with IGT/T2DM and NASH, the improvement in metabolic/histologic parameters by PIO, and in particular the reduction of liver fat content and VF, have a good correlation with the increase in plasma adiponectin levels. This suggests that changes in plasma adiponectin concentration may play an important role in mediating the beneficial effects of PIO in patients with NASH.
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OP 47

Highlights in genetics and prediction of diabetes

0257

Association of KLF11 with insulin resistance is mediated via STAT3 binding to KLF11 variant −1659 G>C

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Background and Aims: Recently, it has been shown that the TGF-β inducible transcription factor, KLF11, affects transcriptional regulation of the insulin gene. Genetic variants of the KLF11 gene are associated to type 2 diabetes (T2D), including the Q62R and promoter −1659 G>C (p-SNP) that are in complete LD. We aimed to study the T2D-association of KLF11 variants by genotyping a general Danish population and analyzing transcriptional regulation of KLF11 affected by p-SNP. This SNP is situated in a putative RBP-Jκ or STAT3 binding site ((G>C) TGGGAA). The transition of G- to C- allele of p-SNP is predicted to result in a loss of transcription factors binding. RBP-Jκ plays an important role in the Notch signaling pathway that enhances differentiation of the endocrine pancreatic beta-cells. On the other hand, STAT3 is necessary for regulation of gluconeogenesis in hepatocytes.

Materials and Methods:We genotyped Q62R in 4,443 normal glucose tolerant (NGT), 491 impaired fasting glucose, 679 impaired glucose tolerance, 251 newly identified T2D and 118 subjects with history of T2D (INTER99 cohort) and analysed association with glucose related-traits. We analyzed nuclear proteins binding onto the p-SNP sequence by gelshift experiments (EMSA). We cloned the KLF11 promoter region containing wild type or mutant p-SNP in a pGL3-luciferase construct and cotransfected them with pCMV-RL, whereafter relative luciferase activity was measured.

Results: In NGT subjects the 62R-allele was a significantly associated with higher fasting serum insulin levels, higher fasting serum C-peptide levels and increased HOMA insulin resistance indexes (P=0.00004, P=0.006 and P=0.00002, respectively). We showed that a protein complex binds onto the wild type p-SNP sequence and that it is absent on the mutant sequence. Our results proof that STAT3 is present in this complex. The analysis of KLF11 promoter activity showed an inhibition of the G-allele compared to the C- allele, suggesting that p-SNP affects KLF11 expression levels.

Conclusion: KLF11 is associated to insulin resistance in the Danish population. The fact that p-SNP alters STAT3 binding and repression of the KLF11 promoter, suggests that KLF11 regulation plays an important role in liver glucose sensibility, which could explain the association of KLF11 with insulin resistance.

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0258

Modulation of genetic susceptibility by behavioral factors and glucose regulation status in the general population exemplified by the impact of the *APOA5* −1131T>C variant on serum lipids

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Background and Aims: Previous studies suggest that the C-allele of the *APOA5* −1131T>C polymorphism confers an increase in serum triglyceride concentrations. The aim of the present study was at the population level to estimate the effect of the *APOA5* −1131T>C polymorphism on fasting serum lipids and to elucidate the potential modulating impact of health behavior and glucose regulation status.

Materials and Methods: The *APOA5* −1131T>C polymorphism (rs662799) was genotyped in a population-based sample (Inter99) of 5,873 treatment-naïve Danes (2,929 men, 2,944 women; age 46±8 years; BMI 26.3±4.6 kg/m^2) using a chip-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of PCR-generated primer extension products.

Results: The *APOA5* −1131T>C polymorphism was associated with dyslipidaemia (defined according to WHO) in a case-control study of 1,439 patients with dyslipidaemia and 4,432 normolipidaemic subjects, $P=4\cdot10^{-6}$ for minor allele frequency (8.2% [7.2–9.2%] vs. 5.7% [5.2–6.2%]) and $P=1\cdot10^{-5}$ for genotype distribution. Correspondingly, in the total population of 5,873 participants the C-allele was associated with increased fasting serum levels of triglyceride ($P=1\cdot10^{-15}$) and total cholesterol ($P=0.0003$) and decreased fasting serum HDL-cholesterol ($P=0.004$). Significant epistasis on fasting serum triglyceride concentrations was observed between the *APOA5* −1131T>C polymorphism and smoking ($P=0.0003$), daily alcohol intake ($P=0.005$), and dietary monounsaturated fatty acid intake ($P=8\cdot10^{-6}$). The effect of the polymorphism on fasting serum triglyceride was more profound in people with impaired glucose regulation than for glucose-tolerant subjects ($P=5\cdot10^{-9}$).

Conclusion: At the level of the general population of middle-aged people the common C-allele of the *APOA5* −1131T>C polymorphism greatly influences the fasting serum lipid levels. Importantly, the impact of this gene variant on fasting serum triglycerides is strongly modulated by behavioral factors. Similarly, the *APOA5* variant effect is much more profound in subjects with hyperglycaemia.

0259

Predictive value of diabetes-associated autoantibodies among children with HLA-conferred disease susceptibility recruited from the general population

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Background and Aims: There is a shortage of data on the predictive value of diabetes-associated autoantibodies in the general population, from which about 90% of new cases

affected by type 1 diabetes (T1D) are derived. We aimed at assessing the predictive characteristics of ICA in combination with IAA, GADA, and/or IA-2A among children with HLA-conferred disease susceptibility recruited from general population.

Materials and Methods: We observed from birth 7412 participants (3513 boys; 47.4%) in the Finnish Type 1 Diabetes Prediction and Prevention study (DIPP) for progression to T1D for an average of 5.6 years (0.9–10.4 years). All subjects carried HLA genotypes conferring increased T1D risk. Blood samples were taken with an interval of 3–12 months throughout the follow-up period. ICA were used as the primary screening tool. If a subject tested positive for ICA, IAA, GADA and IA-2A were measured from all samples available from that individual. Subjects were categorised according to their autoantibody profile in such a way that each child could belong to only one category. Persistent antibody positivity was defined as positive results in at least two consecutive samples including the last sample available. ICA were detected with conventional immunofluorescence, and the other autoantibodies were analysed with specific radiobinding assays.

Results: 763 children (10.3%) tested positive for ICA at least once, 437 of whom (5.9%) were positive for ICA only, 100 (1.3%) for two antibodies, 72 (1.0%) for three antibodies and 154 (2.1%) for all four autoantibodies. Persistent isolated ICA positivity was seen in 287 (3.9%) children, while 75 (1.0%) subjects were persistently positive for two antibodies, 82 (1.1%) for three antibodies and 74 (1.0%) for all four antibodies. 97 (1.3%) children presented with clinical T1D at a mean age of 3.9 years (0.9–9.2 years). The highest disease sensitivity [52.6% (CI_{95} 42.3–62.8%)] was observed for positivity for all four antibodies, while persistent positivity for the combination of ICA+IAA+IA-2A resulted in the highest specificity [100% (CI_{95} 99.9–100%)] and positive predictive value [62.5% (CI_{95} 24.5–91.5%)]. In the survival analyses the highest progression rate was seen in those persistently positive for ICA+IAA+IA-2A, among whom all children observed for more than 7 years (n=5) presented with T1D. Among the children persistently positive for ICA+IAA all the affected children (7/15) were diagnosed before the age of 2 years.

Conclusion: It is possible to identify a cohort of children from the general population with as high a disease risk as that seen among siblings positive for multiple autoantibodies by screening genetically susceptible children for autoantibodies. The high-risk cohort is small, and the disease sensitivity of high-risk antibody markers remains below 55%. However, based on autoantibody markers one can sort out a subgroup of children with rapid progression to T1D and another group in which almost all children appear to progress to overt T1D before the age of 8 years.

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0260

Functional impact of the vitamin D receptor gene *Fok* I polymorphism on immune cells

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Background and Aims: The active form of vitamin D, 1,25-Dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), has potent immunomodulatory properties and is able to prevent autoimmune diabetes in NOD mice. These effects of 1,25 $(OH)_2D_3$ are mediated through binding with the vitamin D receptor (VDR). Several polymorphisms of the human VDR gene have been identified of which the *Fok*I polymorphism results in a VDR protein with a different structure, a long *f*-VDR or a 3 amino acids shorter *F*-VDR. Multiple studies have tried to correlate this VDR *Fok*I polymorphism with the genetic predisposition to type 1 diabetes but failed to reveal a solid association or a functional explanation for the observed correlation. The aim of the present study was to investigate the functional consequences of the *Fok*I polymorphism in immune cells.

Materials and Methods: Transfection experiments with a luciferase reporter construct under control of different promoter sequences were performed in the presence of the long *f*-VDR or short *F*-VDR. Functional experiments were performed on monocytes and lymphocytes isolated from human subjects with a homogenous long *ff* or short *FF* VDR-genotype. IL-12p40 and p35 mRNA expression levels were determined by quantitative real time RT-PCR in IFN-γ-stimulated monocytes. Proliferation of PHA-stimulated lymphocytes was assessed by thymidine incorporation.

Results: In the transfection experiments, the presence of the shorter *F*-VDR resulted in higher NFκB- and NFAT-driven transcription as well as higher transcription driven by the endogenous promoter of IL-12p40. No differential effects were observed for AP-1-driven transcription or transactivation of the classical mouse osteopontin DR3-type vitamin D responsive element. Concordantly, in monocytes from subjects with a homogenous short *FF*

VDR-genotype, expression of IL-12p40 and p35 after *in vitro* IFN-γ stimulation was higher than in monocytes expressing the long *ff* VDR-genotype. Additionally, lymphocytes with a short *FF* VDR-genotype proliferated stronger in response to phytohemagglutinin.

Effects of the VDR FokI polymorphism on immune cell behavior

	long ff genotype	short FF genotype
p40	1.1±0.5	3.8±0.7 (p<0.05)
p35	8.5±3.2	62.8±18.4 (p<0.05)
proliferation (SI)	26.6±12.6	70.1±13.6 (p=0.05)

Conclusion: In his study we have demonstrated clearly that different *Fok*I genotypes interact differently with transcription factors that are important in the immune system. The observed differences between the *Fok*I genotypes at transcriptional level were reflected in a different immune behavior of monocytes and lymphocytes. Together these data provide the first functional evidence that the VDR *Fok*I polymorphism affects immune cell behavior, with a more active immune system for the short *F*-VDR, thus possibly playing a role in autoimmune diseases.

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OP 48

Clinical immunology

0261

Ii-key/MHC class II epitope hybrid technology and ELISPOT assay in type 1 diabetes

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Background and Aims: Type 1 diabetes (T1D) is the result of the immunomediated destruction of insulin producing β cells in the islets of Langherans. This immune response involves both cellular and humoral changes that persist over a prolonged period up to diagnosis. Autontibodies have become a useful marker for the prediction of the disease. Less is known on the specificity of cellular responsiveness and on T-cell profiling in T1D. MHC class II epitopes are normally bound to MHC class II molecules in a post-Golgi, antigenic peptide-binding compartment of antigen presenting T cells. The shortest sequence (Ii-key peptide) (LRKM) of the Ii protein acts at an allosteric site on MHC class II molecules to facilitate charging and presentation of peptides into the antigenic binding site, entirely circumventing the normal process of antigen phagocytosis, processing and intracellular charging onto MHC class II molecules. This offers us an efficient way to augment the binding of epitopes to the antigenic peptide-binding groove, thus enhancing the immunogenecity of epitopes. These observations have been supported by studies in cancer and infectious diseases. The main objective of this study was to investigate the cellular immune response in T1D by characterizing the cytokines secreted by peripheral blood mononuclear cells (PBMCs) in response to the exposure to Ii-key/MHC class II insulin epitope hybrids.

Materials and Methods: Sequences of the human insulin were obtained from Genebank. Predicted DRB1*0301 and DRB1*0401 epitopes were identified by application of the Rammensee SYFPEITH program (http://syfpeithi.bmi-heidelberg.com/Scripts/MHCServer.dll/EpPredict.htm). Five peptide series (15 hybrids and five epitope-controls) were analyzed. The study was carried out in 31 patients (20 M) affected by T1D, and in 10 normal subjects. Diagnosis was defined according ADA classification. PBMCs were prepared from heparinised blood using standard Ficoll Hypaque separation. ELISPOT assays were performed with BD Pharmingen set for IFN-γ according to the manufacturer's instructions. Digitalized images of spots were analyzed with an E*li.Analyse 4.0 software*.

Results: All assays detected significant cytokine secretion in response to the polyclonal stimulators PMA/Ionomycin. Six out 31 patients (19.4%) demonstrated a positive response to one or more peptides. Control subjects (4/10 subjects were DR3 or DR4) showed no detectable response to stimulation with Ii-key hybrids or peptides. Tested peptides were recognized only by DR3/DR4 0302+ve diabetic patients.

Conclusion: The Ii-key hybrid technique is a new strategy for augmenting the potency of MHC class-II restricted epitopes resulting in a profound increase in T cell stimulation. Our study has demonstrated that HLA is crucial for the response to Ii-Key hybrids and this can help to better understand epitope specificity in T1D. Since extensive studies have confirmed the role of ELISPOT assay to detect early antigen-directed activation of lymphocyte subpopulations in T1D, the combined use of the key-hybrids technique and the ELISPOT assay assures a more selective and sensitive cytokine detection in response to

antigen stimulation and could advance our understanding of the cell mediated immune response in T1D.
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0262

Dietary eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) intake is associated with decreased risk of islet autoimmunity in children at risk for type 1 diabetes
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Background and Aims: Studies have suggested that fish oil supplementation in infancy may protect against type 1 diabetes, but little is known about the role of fish oil after infancy on risk of type 1 diabetes or its precursors. We examined whether childhood intake of EPA and DHA, the omega-3 fatty acids found in fish oil, is associated with the development of islet autoimmunity (IA) in children in the Diabetes Autoimmunity Study in the Young (DAISY).
Material and Methods: DAISY is following children who either possess a higher risk HLA genotype or are a first degree relative of someone with type 1 diabetes, for development of IA, which is defined as being positive for insulin, glutamic acid decarboxylase or IA-2 autoantibodies on at least two consecutive occasions and still positive or diabetic at last follow-up. Data on omega-3 and omega-6 fatty acid intake were collected annually starting at the age of 1, using the Willett food frequency questionnaire. To validate the reported data on fatty acid intake, we measured erythrocyte membrane fatty acid levels in 314 children, and found a strong statistically significant association between reported EPA and DHA intake and erythrocyte membrane EPA and DHA levels ($p<0.0001$). Of the 1474 children with complete dietary data, 57 children developed IA at a mean age of 4.8 years, and 1417 children were IA negative after a mean follow-up of 5.5 years. Cox proportional hazards models were used, and hazard ratios (HR) and 95% confidence intervals (CI) were calculated based on a standard deviation difference in nutrient intake. We examined four fatty acid intake variables: 1) total omega-3 fatty acids, 2)EPA&DHA, a summed intake of the two omega-3 fatty acids found in fish oil, 3) total omega-6 fatty acid intake, and 4) arachidonic acid, an omega-6 fatty acid.
Results: Adjusting for HLA DR3/4 status, family history of type 1 diabetes, maternal age, caloric intake, and age at introduction of cereals in the infant diet, EPA&DHA intake was inversely associated (HR: 0.35, CI: 0.16–0.78), and arachidonic acid intake was directly associated (HR: 1.83, CI: 1.12–3.00) with risk of IA. In separate models, total omega-6 fatty acid intake was not associated with risk of IA; and total omega-3 fatty acid intake was marginally inversely associated with IA.
Conclusion: We found that childhood intake of EPA and DHA, the omega-3 fatty acids found in fish oil, may lower risk of developing islet autoimmunity in children at increased risk for diabetes.
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0263

Screening for celiac disease in childhood diabetes: is it enough to screen at the time of clinical diagnosis of diabetes?
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Background and Aims: The prevalence of Celiac Disease (CD) in type 1 Diabetes Mellitus (T1DM) is known from several studies and varies from 2.3 to 10%. CD is often asymptomatic or atypical in children with T1DM. Few children have CD before T1DM is diagnosed. Longitudinal studies are also needed to investigate the development of CD in T1DM patients.
Aims: To investigate the prevalence of CD in a cohort of children and adolescents at the onset of T1DM and the occurence of CD during a six year follow up.
Materials and Methods: A total of 300 patients with T1DM were prospectively studied for CD. The study group was a cohort of children and youths under the age of 19 year at six clinical centers for pediatric diabetes in Scania (Skåne), Sweden. The children were diagnosed with diabetes between Januari 1, 1996 and December 31, 2000. We performed repeated serological analyses from the onset of diabetes and annually thereafter during six years. The IgA-antiendomysium antibody (EMA) test was selected as the screening test and patients with a positive test were considered appropiate for a jejunal biopsy. Two patients had been diagnosed with CD before the onset of their diabetes.
Results: The prevalence of biopsy proven CD at the time of clinical diagnosis of T1DM was 4% (12/300). During the follow up a total of 17 patients developed CD as follows: 10 after one year, 5 after two years, 1 after three years and 1 after five years. The cumulativ frequency of CD therefore represented 29/300 (9,7%), all confirmed by intestinal biopsies. At diagnosis of diabetes in patients with CD the mean age was 8,8 year and in patients without CD 9,9 year. The prevalence of CD in patients with T1DM is approximately 20 times higher than in the general population. It was noted that while 12/29 had CD at the time of clinical

onset of T1DM the remaining group of 17 T1DM children developed CD without clinical symtoms.
Conclusion: Our study confirmes a high prevalence of biopsy proven CD in children and youths with T1DM with the overall prevalence of 9,7%. We suggest that children with T1DM should be screened for CD annully, at least for three years with a possible reduction in test thereafter unless the patient show symptoms.
Supported by the Children Diabetes Foundation

0264

Autoantibodies against cytokeratin 19 in patients with type 1 diabetes mellitus
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Background and Aims: Autoantibodies against antigens expressed in exocrine pancreatic cells, such as carbonic anhydrase II and lactoferrin, have been reported in patients with type 1 diabetes mellitus. Citokeratin 19 (CK19) is an antigen expressed in both pancreatic and biliary ductal cells. Autoantibodies against CK19 (anti-CK19) have been found in patients with autoimmune hepatitis. In this study we verify whether CK19 represents a target antigen for autoimmunity in type 1 diabetes.
Materials and Methods: We evaluated the presence of anti-CK19 in sera from 62 patients (39M/23F; age 1–19 years) with duration of type 1 diabetes mellitus <3 months and from 30 healthy controls (13M/14F; age 3–18 years). Serum levels of autoantibodies anti-CK19 of the IgG class were measured by ELISA and the mean OD +2 SD of controls was considered the upper limit of normal range. Results were expressed as median and range.
Results: Diabetic patients showed anti-CK19 levels higher (0.430, range 0.210–1.690; $p<0.0001$) than control subjects (0.300, range 0.140–0.510). Values above the normal range were found in 23/63 (37%) type 1 diabetic patients and in 1/30 (3.3%) healthy controls ($p<0.001$).
Conclusion: Our results on the presence of anti-CK19 in sera of type 1 diabetic patients represent another evidence of autoimmunity against exocrine pancreatic antigens in type 1 diabetes mellitus.

PS 1

Epidemiology of type 1 diabetes I

0265

Further clues to the aetiology of type 1 diabetes: spatial clustering amongst 0–29 year olds in Yorkshire, UK
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Background and Aims: The aetiology of type 1 diabetes in children and young people has a strong environmental component. The presence of geographical or spatial clustering would suggest localised excesses of relevant environmental exposures. We studied spatial clustering amongst children and young adults aged 0–29 years using population-based data from a specialist register in Yorkshire, UK.
Material and Methods Two data sets of children and young people diagnosed with type 1 diabetes while living in Yorkshire were analyzed: (1) cases aged 0–14 years diagnosed between 1978–2002; (2) cases aged 15–29 years diagnosed between 1991–2002. The Potthoff–Whittinghill method was used to test for spatial clustering.
Results 3019 cases of type 1 diabetes aged 0–14 years and 989 cases of type 1 diabetes aged 15–29 years were analysed. There was statistically significant spatial clustering for 0–14 year olds diagnosed during the period 1978–1985 only ($P=0.009$), mainly involving younger children ($P=0.01$ for 0–4 year olds and $P=0.005$ for 5–9 year olds). There was also significant clustering for 15–29 year olds ($P=0.003$ and $P<0.001$ for cases diagnosed during the periods 1991–1995 and 1996–2005, respectively), especially involving 20–24 year olds ($P=0.002$ and $P=0.01$ for cases diagnosed during the periods 1991–1995 and 1996–2005, respectively).
Conclusion The present study is the first to analyse spatial clustering of type 1 diabetes amongst older teenagers and young adults. The findings show that geographical clustering of cases is present and differs by age and time period. This pattern suggests that environmental factors associated with persistent localised exposure may influence the risk of developing type 1 diabetes in different age groups during different time periods.
Supported by Department of Health

0266

WITHDRAWN

0267

Continued increasing trend of type 1 diabetes incidence in childhood in North Rhine–Westphalia, Germany

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Background and Aims: A large risk population and a sufficiently long observation period are important preconditions for valid estimation of Type 1 diabetes incidence in childhood and its temporal trend. Aim of the present study was to estimate incidence and time trend of Type 1 diabetes in children 0–14 years of age in the large risk population of the German federal state North Rhine–Westphalia (NRW) during 1996–2003.
Materials and Methods: NRW has the largest population among all German federal states comprising about one quarter of all children in the age group 0–14 years in Germany. During the study period the average risk population was 2.91 millions children. The North Rhine–Wesphalian diabetes incidence register ascertains newly diagnosed cases of type 1 diabetes by means of three data sources: the prospective hospital-based active surveillance system ESPED, annual inquiries among paediatric, internal, and general medical practices, and the computer-based documentation system DPV founded for quality control and scientific research in paediatric diabetes care. Completeness of ascertainment was estimated by the capture–recapture-method using log-linear modelling. Point and interval estimates (95%-CI) of incidence rates (per 100,000 person–years) were based on Poisson distribution. Age-and sex-standardized rates were estimated by the direct method using equal weights. Poisson regression analysis was applied to assess time trends.
Results: During 1996–2003 a total of 4,261 newly diagnosed diabetic children aged 0–14 years (2,227 boys, 2,034 girls) were registered. Ascertainment was estimated to be 96.8% (95%–CI: 96.4%–97.2%) complete. The overall age- and sex-standardized incidence rate was 18.1 (17.6–18.7). The age-standardized incidence among boys (18.5, 17.7–19.3) was slightly higher than among girls (17.8, 17.0–18.5, p=0.188). Incidence depended significantly on age (p<0.001). Age-specific estimates for age groups 0–4, 5–9, and 10–14 years were 12.5 (11.7–12.4), 19.8 (18.8–20.8), and 22.0 (21.0–23.1), respectively. Annual incidence rates ranged between 15.4 in 1998 and 21.7 in 2003. The average annual incidence increase was estimated as 3.9% (2.5%–5.3%). There was no significant difference in incidence trend among boys and girls (annual increase: 4.3% vs. 3.4%, p=0.526). But incidence trend varied significantly between age-groups (p=0.012), with the steepest increase in youngest children. Annual increases for the age groups 0–4, 5–9, and 10–14 years were 7.7%, 3.8%, and 2.1%, respectively.
Conclusion: These data based on a large risk population and high case ascertainment confirm that the incidence of type 1 diabetes in childhood is steadily increasing in Germany. The annual increase was slightly higher than the average increase in Europe. In accordance with other European data the steepest rise was observed among youngest children. Based on the observed incidence rate, there are annually about 2,500 children newly diagnosed with type 1 diabetes in Germany, what underlines the public health importance of childhood diabetes care. Further research is needed to identify causes of the continuous rise of diabetes incidence.
Supported by German Ministry of Health, Ministry of Innovation, Science, Research and Technology of the State of North Rhine–Westphalia

0268

The parallel increase of type 1 diabetes incidence in two different regions of Poland: highly industralised Upper Silesia and agricultural North–Eastern regions of Podlasie and Warmia–Mazury

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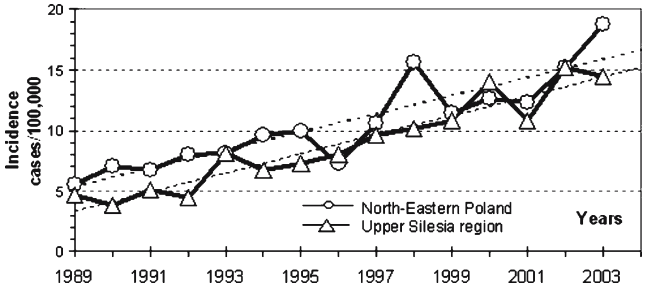
Background and Aims: The incidence of childhood type 1diabetes in Poland has been increasing, at least since the early 1970's (3.5/100.000 in 1970–1981 vs. 6.6/100.000 in 1982–1985). A number of subsequent studies from several regions of Poland have suggested a further increase, but the reasons remain unknown.
Material and Methods: The aim of the present study was to evaluate if the increase of T1D incidence could be related to the

environmental factors. Upper Silesia is the most industrialized, urbanized and polluted part of Poland, in contrast to the Podlasie and Warmia–Mazury regions — forested and agricultural. In both regions data concerning new cases of T1D among children aged 0–14 years were collected prospectively between 1989 and 2003 as a part of EURODIAB program.

Results and Conclusions: The most recent data shows that in both regions the incidence of T1D has reached levels typical to Western Europe (14.5/100.000 in Upper Silesia and 18.8/100.000 in North–Eastern Poland). The average annual incidence increases since 1989 in Upper Silesia (8.4%/y) and North–Eastern Poland (7.0%/y) suggest that the epidemic of T1D has more features of a secular trend than year-to-year outbreaks. In addition, the factor(s) driving the epidemic are apparently independent of the environmental pollution and currently unabating in Poland.



0269

Environmental risk factors affecting high incidence rate of type 1 diabetes in north–eastern Poland

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Background and Aims: In north–eastern Poland, Warmia and Mazury Region (W&M Region) an increase in the incidence rate (IR) of type 1 diabetes (T1DM) was observed in the years 1994–2003**.** The annual IR increase in the age group 0–29 was 4.6%, in this 9.8% in the age group 0–14. The aim of the study was to assess the influence of selected environmental risk factors on the T1DM IR.

Materials and Methods: The prospective register was carried on in W&M region according to EURODIAB methods. Three hundred thirty-one patients aged 0–29 were registered in the years 1994–2003. Perinatal, alimentary, infectious, socio-economic, climatic and pollution risk factors were assessed. The data obtained were compared with the data characteristic for the general Polish population.

Results: Type 1 diabetes IR was closely associated with urban area residence compared to rural area; i.e. 12.7/100 000 (95%CI:10.4–15.0) and 8.8/100 000 (95%CI:6.7–10.9) ($p<0.05$), respectively, but only for children aged 0–14 years. The place of residence was not a risk factor for the older age groups. The age of mothers over 25, and especially over 35 years, was significantly associated with increased risk of T1DM ($p<0.05$). Diabetes was diagnosed more often in children born from the second and the following deliveries compared to the first one ($p<0.05$). The duration of full breast-feeding shorter than 3 months and early exposure to cow's milk was associated with higher IR of T1DM in our region ($p<0.05$). Seasonality was reflected in higher IR of T1DM in autumn and winter than in summer; i.e. 1.2/100,000 vs 0.2/100,000 ($p<0.0$), respectively. The secondary and higher education level of mothers of patients with T1DM proved high risk factors ($p<0.05$). No connection was found between birth weight, caesarean delivery, viral infections (mumps, roseola, chicken-pox), vaccinations, water composition and pollution after the Chernobyl accident and the incidence of T1DM in our region.

Conclusion: Among the studied risk factors for T1DM the significant ones were:
–place of residence
–age of mother
–number of deliveries
–full breast feeding period shorter than 3 months
–seasonality of incidence
–hygienisation of life resulting from mother's education level

0270

New data regarding the influence of gender and ethnicity on the incidence of childhood type 1 diabetes mellitus in Romania

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Background and Aims. Romania is a country having a low/intermediate incidence of type 1 diabetes mellitus (T1DM) in children. As in other countries, the number of new cases of T1DM is getting higher in Romania, too. The purpose of this work was to assess the time trends in the incidence of childhood T1DM and to reveal the influences determined by gender and ethnicity.

Material and Methods. Data were obtained from two sources: 1. Romanian Childhood Diabetes Registry, developed since 1995 by ONROCAD (Romanian acronym for "Romanian National Organization for the Protection of Children and Adolescents with Diabetes"); 2. Medical records from Clinical Center "Cristian Serban" Buzias,

where children with T1DM from Romania are treated. Till year 2001, only data from children aged 0–14 years were obtained. Starting year 2002, ONROCAD collected data from age group 0–18 years, too

Results. Table 1 presents the incidence of T1DM in children from Romania, between years 1996 and 2005.

Table 1

Incidence*		1996	1997	1998	1999	2000	2001	2002	2003	2004	2005
General (0–14 years)		3.91	4.02	4.10	4.97	5.05	5.66	4.88	5.47	5.36	6.30
Gender	**G**	3.96	4.68	4.34	5.23	4.94	5.04	4.34	5.41	4.93	6.21
(0–14 years)	**B**	3.88	3.39	3.72	4.56	5.15	6.15	5.34	5.52	5.78	6.39
Ethnicity	**R**	–	–	3.90	4.57	4.82	5.53	4.64	5.23	5.38	6.30
(0–14 years)	**H**	–	–	7.02	8.98	7.88	7.19	5.91	11.63	9.77	8.84
General (0–18 years)		–	–	–	–	–	–	5.51	5.67	5.93	6.12
Gender	**G**	–	–	–	–	–	–	4.71	5.31	5.23	5.87
(0–18 years)	**B**	–	–	–	–	–	–	6.28	6.01	6.66	6.36
Ethnicity	**R**	–	–	–	–	–	–	5.34	5.62	5.93	6.13
(0–18 years)	**H**	–	–	–	–	–	–	8.27	9.31	10.69	8.27

Legend: *=no. of cases/100,000; G=girls; B=boys; R=Romanians; H=Hungarians.

Some remarks can be made: 1. The general incidence in age group 0–14 years increased with 61% between 1996 and 2005. A plateau was registered between years 2001 and 2004. 2. Incidence in age group 0–18 years increased from 2002 to 2005, especially in girls. 3. The incidence was higher in girls between 1996 and1999 and in boys thereafter. 4. The incidence in Romanians was lower than in Hungarians (the main ethnic minority); environmental factors being the same, the only explanation is given by genetic differences.

Conclusions. The incidence in T1DM in Romania showed an increasing trend over the past 10 years. The girls were more frequent affected in the first half of the decade and the boys thereafter. Children from the Hungarian minority have a higher incidence than those from the Romanian majority, due to their genetic background.

PS 2

Epidemiology of type 1 diabetes II

0271

Factors affecting Body Mass Index from onset of diabetes to age 18

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Background and Aims: The Danish Childhood Diabetes Register was founded in 1996. It is a nation-wide register collecting data from all Danish paediatric diabetes centres treating type-1-diabetic patients aged 0–15 years. Weight and height is measured at onset of the disease and yearly thereafter. The aim of the present study was to describe the factors affecting body mass index (BMI) from onset to age 18.

Materials and Methods: Weight and height is measured by health professionals at each centre. Weight is measured in kilogram, height in m. Weight and height measured less than 30 day after onset were excluded. BMI was calculated as weight **(kg)**/height **(m)**^{2} In the model BMI was entered as sds–score. Standard deviation score (SDS) was defined as (observed – expected)/standard deviation for age and sex using a Danish reference material. General linear modelling was used to estimate the association between the different risk factors and the BMI sds.

Results: There are 2906 children in the register, and 13310 year reports with height and weight data. There was a significant increase in BMI sds with length of diabetes ($p<0.01$) and age of onset ($p<0.01$). There was a significant interaction between diabetes duration and sex. Girls tended to start on a lower level, but had a more steep increase in BMI. The BMI sds were the same for boy and girls after approximately two years of diabetes, thereafter the girls tended to have a higher BMI sds. The BMI sds differed between centres. There were a significant association between BMI sds and HbA1c and centre. There were no association with year of onset.

Conclusion: BMI sds differed between centres and was associated with diabetes duration, sex, HbA1c and age at onset.

0272

Breastfeeding among mothers with type 1 diabetes (T1D) in a primary diabetes prevention trial

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Background and Aims: Difficulties in initiating and maintaining breastfeeding among mothers with T1D have previously been reported. The aim of this study was to

compare breastfeeding among mothers with and without T1D participating in the Trial to Reduce IDDM in the Genetically at Risk (TRIGR).

Materials and Methods: The present analysis included 696 mothers with and 690 mothers without T1D, whose infants were at least 9 months old by the end of February 2006. Information on breastfeeding was acquired from the family by dietary interviews conducted at 2 weeks and at 1, 2, 3, 4, 5, 6 and 9 months of age of the infant.

Results: During the first 2 weeks of life 89% of the mothers with T1D breastfed their infants versus 93% of the mothers without T1D. At 3, 6 and 9 months the proportions of infants being breastfed were significantly different ($p<0.001$) with 65, 50 and 38% among the mothers with T1D and 82, 71 and 53% among the other mothers, respectively. Median duration of exclusive breastfeeding was 0.1 weeks and 1.4 weeks among mothers with and without T1D, respectively ($p<0.001$).

Conclusion: The proportion of mothers with or without T1D who initially breastfed their infants did not differ significantly. However, the duration of both exclusive and total breastfeeding was shorter among the mothers with T1D.

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0273

Seasonal variation of newborns with cord blood islet autoantibodies

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Background and Aims: Genetic factors and unknown environmental factors are important in the development of islet autoimmunity and Type1 Diabetes (TID). The aim of this study was to test whether autoantibodies against GAD65, IA-2 and insulin, which predict type 1 diabetes, in the cord blood of newborns born to non-diabetic mothers may mark a triggering event during pregnancy.

Materials and Methods: HLA-DQ genotypes were determined in dried blood spots taken at birth from more than 35,000 children in the Diabetes Prediction in Skåne (DiPiS) study. Autoantibodies to glutamic acid decarboxylase (GAD65Ab), islet associated antigen-2 (IA-2Ab), and insulin (IAA) were determined in standard radioligand binding assays. Information about the pregnancy was obtain at birth by the midwife and from a questionnaire administered to the mother when the child was two months of age. Newborns of mothers reporting they had diabetes (T1D or T2D) or gestational diabetes at birth were excluded from this study.

Results: A total of 1.6% (GAD65Ab, 0.58%; IA-2Ab 0.06%; IAA, 1.0%) newborns had high levels of islet autoantibodies in the cord blood. Cord blood serum levels of GAD65Ab ($r=0.96$, $p<0.001$) and IA-2Ab ($r=0.90$, $p<0.001$) correlated strongly with levels in the mother at the time of delivery. HLA-DQ2/8 conferring the highest genetic risk of T1D was associated with GAD65Ab (OR 1.98 (95%CI was 1.13–3.49), $p=0.017$), IA-2Ab (OR 4.86 (95%CI was 1.42–16.57), $p=0.011$) as well as IAA (OR 2.20 (95%CI was 1.45–3.34), $p<0.0005$). The prevalence of newborns with islet autoantibodies was increased in the third yearly quarter (July, August and September in four consecutive years): GAD65Ab: OR 1.37 (95%CI was 1.01–1.85), $p=0.046$); IA-2Ab OR 2.09 (95%CI was 0.86–5.10), $p=0.10$; IAA, OR 2.02 (95%CI was 1.63–2.52), $p<0.0001$) compared to the rest of the year. The prevalence of reported gastroenteritis ($p<0.0001$) or fever ($p<0.0001$) during pregnancy also showed a monthly seasonal variation, with a consistent lag time correlation with prevalence of autoantibodies that was strongest at 6–7 months prior to birth ($p<0.001$). Among children born in the third quarter there was a positive association between islet autoantibodies in the newborn and reported gastroenteritis (OR 1.80 (1.04–3.11), $p=0.037$) in the mother. The HLA and environmental associations with cord blood autoantibodies were independently significant.

Conclusion: Autoantibodies detected in cord blood showed consistent seasonal variation over four years. The increased frequency of islet autoantibodies was associated with genetic and environmental association that occurs primarily through the mother. The data suggest that environmental exposure during early pregnancy may trigger islet autoimmunity which may have consequences not only for the risk of the mother to develop type 1 diabetes during or after pregnancy but also for the offspring.

Supported by Lund University

0274

Increased relative birthweight in children with diabetes high risk HLA may be enhanced by infections during pregnancy

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Background and Aims: It has been reported that children born with HLA conferring high risk for type 1 diabetes (T1D) have an increased high relative birthweight (HrBW). The underlying mechanisms for the HrBW is not understood, but gestational infections, islet autoantibodies, or both, are thought to contribute to T1D risk. The aim of this study was to test following hypotheses: 1) Mothers reporting infections during pregnancy give birth to children with HrBW; 2) Infections during pregnancy affect the association between HLA and HrBW; 3) The reduction in HrBW by islet autoantibodies as shown previously is explained by infections during pregnancy.
Material and Methods: HLA-genotypes were determined in dried blood spots of cord blood from children in the population-based Diabetes Prediction in Skane (DiPiS) study. Children born preterm (<37 weeks of gestation) and to mothers with diabetes or gestational diabetes were excluded. Standard radioligand binding assays were used to determine GAD65Ab, IA-2Ab and IAA. Birthweight (BW) was adjusted for gestational age and divided into quartiles. Upper quartile was defined as HrBW. Two months after delivery, the mother completed questionnaires on gastroenteritis, fever or both during pregnancy.
Results: A total of 19.3% (3832/19 756) mothers reported infections during pregnancy. 10.7% reported gastroenteritis with or without fever and 3.7% fever without gastroenteritis. Islet autoantibody negative children born to mothers reporting infections during pregnancy had an increased risk for HrBW, with the highest risk with fever and/or gastroenteritis across several trimesters (OR 1.51 (95% CI was 1.19–1.91), p<0.001). This effect on HrBW was aggravated in children born with the HLA-DQ2/8 or DQ8/X T1D high risk genotypes (OR 2.31 (1.11–4.82), p=0.025) and in the T1D low risk genotype HLA-DQB1*0603 (OR 2.32 (1.14–4.72), p=0.021). Autoantibodies present in cord blood modified the effect of infections by decreasing the risk of HrBW with fever and/or gastroenteritis in one trimester (OR 0.32 (0.17–0.60), p<0.0005) or several trimesters (OR 0.10 (0.01– 0.74), p=0.024).
Conclusion: This study revealed 1) that children have an increased HrBW when born to mothers reporting infections with fever, gastroenteritis, or both, during pregnancy; 2) that the previously reported association between T1D high risk HLA with HrBW is aggravated in children born to mothers reporting infections during pregnancy and 3) that islet autoantibodies in cord blood decreases HrBW if mothers reports infections during pregnancy. These data suggests an interaction between HLA, gestational infections, islet autoantibodies and fetal growth.
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0275

Enterovirus but not adenovirus infections are associated with autoantibody defined risk in children from the general population and in children at onset of the disease

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Background and Aims: Environmental factors as virus infections have been associated with the manifestation of type 1 diabetes (T1DM) in several but not all studies performed so far. As shown for autoantibody (AAb) positive first degree relatives of patients, the risk to develop T1DM is also increased in probands from the general population if multiple AAbs against different beta-cell antigens occur. The aim was to study if enterovirus (EV) and adenovirus (ADV) infections are associated with the risk to develop T1DM in AAb positive children from the general population and in children at onset.
Materials and Methods: EV-RNA and ADV-DNA sequences were detected by conventional RT-PCR as well as by two-step TaqMan RT-PCR in fifty healthy schoolchildren without heredity of T1DM recruited from the Karlsburg type 1 diabetes risk study and differentiated according to their AAb profile in the first or second follow-up serum sample into three risk groups: 29 probands at high risk (HR; >1 AAb), 10 at moderate risk (MR; 1 AAb at high titer) and 11 at low risk (LR; 1 AAb at low titer). Furthermore, 47 children with newly diagnosed T1DM (median diabetes duration 5 days) and 50 AAb negative control children were involved.
Results: The overall prevalence of EV-RNA amounted to 20% (10/50) in AAb positive children and 36% (17/47) in children at onset and was significantly increased comparison to controls (4%; 2/50; p=0.03 and p<0.001, respectively). However, if the prevalence of EV-RNA is analyzed according to AAb defined risk, only children at HR have significantly increased frequencies of virus infections in comparison to controls: 24.1% (7/29, p=0.02) in RT-PCR and 28.6% (8/28, p=0.002) in two-step RT-PCR. The prevalence of EV-RNA in children at MR or LR did not differ from those of controls. Characterization of the EV amplicons by direct sequencing revealed homology of 66.7% (8/12) with coxsackievirus B group (B4: n=5, B2: n=2, and B6: n=1) in the HR group and of 50% (10/20) with coxsackievirus B4 in probands at onset. During the

follow-up of the study, 12 children at HR (four tested positive for EV-RNA) and one child at MR have progressed to clinical T1DM so far. For ADV no data supporting an association with T1DM have been found; ADV-DNA was detected in 14% (7/50) samples of AAb positive children, in 2.1% (1/47) samples at onset children and in 4% (2/50) of controls. There was no significant seasonality of sampling in any of the groups investigated. However, EV-RNA was found significant frequently in 55% (11/20) of children at onset in autumn and winter (September to February) in comparison to 22.2% (6/27) of children at onset in spring and summer (March to August; p=0.046).
Conclusion: Our data demonstrate that EV infections but not ADV infections are a risk factor for T1DM and are associated with the induction of beta-cell autoimmunity before the onset of the disease. Especially in genetically susceptible probands at high risk identified by combined AAb screening from the general population, EV infections seems to accelerate and/or initiate the beta-cell destruction.
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0276

Activity of constitutive, antiviral enzyme (2′5′-oligoadenylate synthetase) is significantly correlated with GADA levels in T1D families

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Background and Aims: During the pathogenesis of Type 1 diabetes (T1D), the processes that initiate damage to the β cells in the islets of Langerhans remain unknown. Increasing evidence indicates that infection with pancreotropic viruses could be one of the first events that initiates the pathogenic course, ultimately leading to eradication of the β ells. In our human studies of the innate, antiviral immune defense in T1D, we have found that activity of the antiviral, IFN-α inducible enzyme, 2′5′-oligoadenylate synthetase (2′5′AS) is significantly and persistently increased in T1D patients. We have demonstrated that 2′5′AS activity is under strong genetic control by specific genotypes in the *OAS1* (2′5′AS) gene, implicating a possible role for 2′5′AS in the pathogenesis of T1D. The total, constitutive 2′5′AS activity would result in induction of apoptosis and reduction of the β cell mass. GADA (glutamic acid decarboxylase autoantibodies) is a widely used marker for identifying subjects at risk of developing T1D. The appearance of GAD epitopes reflects a process where β cells are dysfunctional or are undergoing apoptosis. Whether these processes are caused by latent virus infections or β cell specific intrinsic factors are not well understood. We propose that constitutive 2′5′AS activity could be a contributing factor in β cell killing. The aim of the study is to determine a possible association between constitutively expressed 2′5′AS enzyme activity and the levels of GADA in individuals with T1D.
Materials and Methods: The study group was Danish nuclear families. In these families there were 82 diabetic and 82 healthy siblings. GADA levels were determined according to international standards. Cutoff level chosen for autoantibody detection was 70 U/ml. Three extreme GADA values, 1–2 orders of magnitude higher than mean values, were excluded from calculations. Activity of 2′5′AS was analyzed in a radiometric assay, in which 2′5′AS catalyzes the conversion of ATP to 2′5′- oligoadenylates of different chain-lenghts.
Statistical testing was done by correlation and regression analyses.
Results: In siblings with T1D, 49% (40/82) were positive for GADA. In healthy siblings, 7.3% (6/82) were positive for GADA. Totally, 46 individuals were found positive for GADA. Of these, six individuals were not having T1D. When the levels of GADA in the included individuals (n=43) were tested against 2′5′AS activity levels, we found a significant correlation, r=0.43, p=0.004. These results indicate a high degree of co-variation between the two parameters.
Conclusion: Previously we have shown that activity of the antiviral enzyme 2′5′AS is under strong genetic control, a finding that is of interest in studies of pathogenesis of T1D because 2′5′AS activity induces apoptosis. Functionally, the enzyme plays two roles, induction of a constitutive, "physiological" apoptosis in β cells and/or induction of very high levels of apoptosis when the enzyme becomes activated during virus infections. In both cases β cells will die to different degrees. It is suggested that a genetically controlled 2′5′AS activity might facilitate β cell killing and lead to generation of GADA in predisposed individuals.
Supported by JDRF, NIH and Children's Hospital Foundation, Vancouver

0277

Serum adiponectin and the development of microangiopathy in type 1 diabetes: An inception cohort Study

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Background and Aims: Low circulating adiponectin (ADPN) levels have been associated with an increased risk of cardiovascular disease and retinopathy in patients with type 2 diabetes. In contrast, several studies have observed elevated serum ADPN levels in patients with type 1 diabetes. Furthermore, a recent cross-sectional study from our group showed even higher serum ADPN levels in type 1 diabetic patients with microvascular complications as compared to matched counterparts without complications. Accordingly, we wanted to investigate the potential predictive value of serum ADPN for the development of microvascular complications in type 1 diabetes.

Materials and Methods: A cohort (n=270) of newly diagnosed type 1 diabetic patients was followed for 18.0 years (median; range 1.0–21.8 years). Baseline levels of ADPN were measured in all participants after 3 years of diabetes. A second ADPN measurement was performed in 206 patients and took place either when they developed a microvascular complication or when the diabetes duration exceeded 15 years. Serum ADPN was measured by a validated in-house sandwich assay.

Results: Baseline serum ADPN levels did not differ between those patients who later developed micro- or macroalbuminuria and those who did not develop diabetic nephropathy (P=0.5). Similarly, there were no differences in baseline levels of ADPN between patients who developed diabetic retinopathy (simplex as well as proliferative) and those who did not (P=0.3). When patients were stratified according to baseline serum ADPN levels above or below the median and analyzed for the time to develop simplex retinopathy, there was a tendency towards a protective role of high ADPN levels (P=0.05).

Conclusion: Despite of previously reported increased levels of serum ADPN levels in type 1 diabetic patients with microvascular complications, we found no significant predictive value of serum ADPN in an inception cohort study comprising 270 type 1 diabetic patients with an median follow-up of 18 years.

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PS 3

Genetics of type 1 diabetes

0278

T1DBase: Integration of public and private genetics, genomics and functional data in type 1 diabetes

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Background & Aims: T1DBase (http://T1DBase.org) is a website and database that supports the type 1 diabetes (T1D) research community. T1DBase is a collaboration between the JDRF Center for Bioinformatics at ISB and the JDRF/Wellcome Trust (WT) Diabetes and Inflammation Laboratory (DIL) at the University of Cambridge. The Beta Cell Gene Expression Bank at T1DBase is the result of collaboration with D. Eizirik (Laboratory for Experimental Medicine, Free University of Brussels). We are also functionally interfacing T1DBase with EPConDB (Beta Cell Biology Consortium). Our aim is to integrate T1D specific and general biological data to create an overview of ongoing research and to provide informatics and genetics tools to T1D researchers in order to promote collaboration by allowing researchers to compare data and share results.

Methods: T1DBase focuses on the molecular genetics and biology of T1D susceptibility and pathogenesis. T1DBase contains data from human, mouse and rat. Datasets include: annotated genomes, genetically identified T1D susceptibility regions and genes, genotypes from the recently published Quad-Scan and other studies, descriptions of NOD mouse congenic strains (with L. Wicker, JDRF/WT DIL), tissue-specific gene expression, microarray data from published experiments, protein interactions from public databases, and biological pathways from KEGG and BioCarta. Data for a given gene is presented on a single page, using well-defined orthology. Tools include the GBrowse genome browser, site-wide search, Connect-the-Dots for connecting gene identifiers, Cytoscape for biological networks, and a microarray viewer.

Over the last year, we continued to develop new tools, integrate new data sources, and seek new collaborations. T1DBase includes gene models from Ensembl, EPConDB, CCDS and DIL and provides consensus models. The Beta Cell Gene Atlas provides beta cell gene expression under different conditions. Using this data, we have compiled a list of genes that are expressed in the beta cell under basal conditions. The Beta Cell Gene Expression Bank contains over 750 beta cell-specific gene annotations and curated beta cell-specific pathways.

Marker data from dbSNP and HapMap, including genotypes, has been added and is available through a BioMart-style filter. The HapMap and dbSNP data has been extracted and imported into GLIDER (Genetics Laboratory Interactive Database and Experimental Repository). GLIDER provides a generic data store for all genotype data and can be easily expanded to contain additional datasets.

My T1DBase allows users to create folders of genes, markers or other features of interest. These collections can be used as a starting point for tools and to save state for future visits.

In the T1DBase Gene Dossier, a list of genes is scored across a number of T1D specific datasets, including pathways and interaction networks.

Conclusions: T1DBase has approximately 500 visits per day (a visit is a continuous session by a single user and generally involves many page hits), indicating that the system is widely employed. We aim to extend its utility to all T1D research areas from DNA sequence to disease mechanism.

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0279

Better Diabetes Diagnostic (BDD): A country-wide registry of incident patients suggests an altered HLA genotype distribution from 1986–87

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Background: HLA genotypes among new onset type 1 diabetes patients is changing overtime and that there is an increased frequency of type 2 diabetes among teenagers. Sweden has the second highest incidence rate in the world and the age at onset has been shown to decrease.

Aim. The aim of the present study was to test the hypothesis that HLA typing at the time of diagnosis would better diabetes diagnosis and improve classification and thereby treatment.

Materials and Methods: The case-control study of all patients developing type 1 diabetes in 1986–87 (n=430) was the baseline reference study available at http://stat-db.stat.sfu.ca/magenta/. The Better Diabetes Diagnosis (BDD) project was initiated in May 2005. Almost all 42 Pediatric clinics in Sweden participate by preparing Dried Blood Spots (DBS) of blood samples taken on the day of clinical diagnosis. HLA DQA1-B1 genotypes were determined by PCR on the DBS using allele specific probes detected by time-resolved fluorescence.

Results: A total of 491 incident patients have been analyzed about nine months into the BDD study. The most common HLA DQA1-B1 genotype, DQB1*0501-0201/0301-0302, which was 36% in 1986–87 had decreased in frequency to 27% (130/491) in 2005–2006. The DQ A1*-B1* genotype 0301-0302/0301-0302 had increased from 8% to 28%. Other significant changes included the 0301-0302/0102-0604 genotype which showed a modest increased from 4% to 6% (31/491). The homozygous genotype 0501-0201/0501-0201 increased from 3% in 1986–87 to 11% (56/491). DQB1*0602 was not present in any child 1968–87 but in BDD 6 0602-positive children have developed diabetes.

Conclusion: It is concluded that there is a temporal change in HLA genotype distribution. The increased frequency of the DQB1*0201/0201 genotype may explain an increasing prevalence of celiac disease at the time of clinical diagnosis of autoimmune diabetes. We speculate that the change in HLA distribution may reflect adaptation of triggering infectious agent(s) to specific HLA.

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0280

HLA-DQ alleles in children and adolescents with type 1 diabetes: temporal changes

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Background and Aims: The incidence of type 1 diabetes presenting in individuals aged 0–19 years in Canterbury (NZ) has increased significantly with time over the last three decades. Ascertainment of all cases is complete for the years 1970 to the present day. Reduction in the prevalence of high-risk HLA genotypes in patients with type 1 diabetes has been reported, against increasing incidence of the disease, suggesting that environmental factors are contributing to increasing disease penetrance. This research investigated the frequencies of HLA-DQ genotypes in individuals with type 1 diabetes diagnosed prior to 20 years of age.
Materials and Methods: All new cases of diabetes aged 0–19 years in the study area were either admitted to the regional hospital or attended acute intervention outpatient clinics at the same institution. Blood samples have been taken at diagnosis for determination of antibodies against islet antigens and for characterisation of HLA-DQ alleles in all consenting individuals diagnosed since 1990. HLA-DQα and β alleles were characterised by PCR-RFLP using group specific primers. The distribution of alleles conferring susceptibility for type 1 diabetes was compared across incidence years
Results: HLA-DQα and β genotypes are available for 252 of the 344 cases diagnosed between 1990 and 2004. The proportion of cases expressing one or two of the HLA-DQα susceptibility alleles, 0301 and 0501, was 37% and 59% respectively. The proportion of cases presenting with two copies of the HLA-DQα susceptibility alleles did not change over time. The HLA-DQβ susceptibility alleles, 0201 and 0302, were present in 32% and 50% of cases respectively. The proportion of cases carrying two HLA-DQβ susceptibility alleles decreased over the study period ($p<0.01$).
Conclusion: The prevalence of HLA-DQβ susceptibility alleles in type 1 diabetes cases diagnosed before 20 years of age has reduced over time reflecting a shift in the relative importance of environmental factors over genetic factors in the development of type 1 diabetes.
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0281

DNA typing in Russian patients with type 1 diabetes mellitus

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Background and Aims: Type 1 diabetes mellitus (T1DM) is caused by the interplay of genetic and environmental factors. Human leukocyte antigen system (HLA) has been demonstrated to contribute to T1DM genetic susceptibility. Tumor necrosis factor α (TNFα) and interleukin 1β (IL-1β) activate inducible NO-synthase in β-cells causing their destruction. 1,25-Dihydroxivitamin D_3 inhibits lymphocyte activation and affects cytokine and immunoglobulin production preventing the development of autoimmune diabetes in NOD mice. Thus, the genes, encoding TNFα, IL-1β and vitamin D receptor can be supposed as candidate genes for T1DM. We aimed to analyze the relationship between *HLA-DRB1* and *HLA-DQB1* genes, *−308G/A* polymorphism of TNFα gene (*TNFA*), *−511T/C* polymorphism of IL-1β gene (*IL1B*), *FokI* polymorphism of vitamin D receptor gene (*VDR*) and susceptibility to T1DM in Russians of Bashkortostan.
Materials and Methods: We PCR-analyzed DNA of 118 Russian T1DM patients and 254 Russian controls living in Bashkortostan. Two-tailed test of Fisher was used for statistical analysis. Bonferroni correction for multiple comparisons was applied during *HLA* polymorphism investigation.
Results: The *HLA-DRB1* (investigated in 99 T1DM patients and 100 controls) and *HLA-DQB1* (analyzed in 100 T1DM patients and 100 controls) genomic typing revealed increased frequencies of *DRB1*04* (33.3% vs. 11.0%, P=0.006), *DRB1*17* (23.7% vs. 9.0%, P=0.006), *DRB1*04/*17* (23.2% vs. 3.0%, P=0.006), *DQB1*0201/*0501* (43.0% vs. 14.0%, P=0.001) in T1DM patients compared to controls. The ratio of the *DRB1*11* (4.0% vs. 15.0%, P=0.006), *DRB1*15* (0.0% vs. 8.0%, P=0.006), *DQB1*0301* (12.5% vs. 25.0%, P=0.020), *DQB1*0602-08* (0.5% vs. 12.0%, P<0.001) were lower in T1DM patients than in controls. Concerning *−308G/A* polymorphism of *TNFA* gene (researched in 116 T1DM patients and 226 controls), we couldn't find any differences of the genotype and allele frequencies between T1DM patients and healthy people. The distribution of the *IL1B* gene *−511T/C* polymorphism *TT* (25.9% vs. 10.1%, P=0.001), *TC* (68.5% vs. 46.8%, P<0.001) and *CC* (5.6% vs. 43.1%, P<0.001) genotypes (examined in 109 T1DM patients and 216 controls) was different among controls and T1DM patients. The typing of *FokI* polymorphism of *VDR* gene (carried out in 118 T1DM patients and 214 controls) revealed that the genotype *ff* is more frequent in controls than in patients (17.8% vs. 8.5%, P=0.023).
Conclusion: Thus, the predisposing markers of T1DM in Russians of Bashkortostan are *HLA* alleles *DRB1*04* (OR=4.05, CI=2.28–6.89), *DRB1*17* (OR=3.15, CI=1.75–5.65), *HLA* genotypes *DRB1*04/*17* (OR=9.79, CI=2.83–33.81), *DQB1*0201/*0501* (OR=3.94, CI=2.00–7.80), genotype *CC* of *IL1B* gene *−511T/C* polymorphism

(OR=8.36, CI=3.10–22.60). The protective genetic markers are *HLA* alleles *DRB1*11* (OR=0.24, CI=0.11–0.53), *DRB1*15* (OR=0.03, CI=0.00–0.47), *DQB1*0301* (OR=0.43, CI=0.25–0.73), *DQB1*0602-08* (OR=0.04, CI=0.01–0.33), genotypes *TT* (OR=0.32, CI=0.16–0.34) and *TC* (OR=0.40, CI=0.25–0.47) of *IL1B* gene *−511T/C* polymorphism, genotype *ff* (OR=0.43, CI=0.21–0.90) of *VDR* gene *FokI* polymorphism.

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0282

Analysis of genetic factors predisposing to type 1 diabetes in children under 5 years of age

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Background and Aims: Type 1 diabetes is a multifactorial disease. The genetic contribution is not straightforward and involves many loci. The strongest association is with HLA region and the results of multilocus analysis provided evidence of between 12 and 20 other susceptibility loci. Recently it has been claimed that the incidence of type 1 diabetes has increased in many countries including Poland. The highest increase occurred in children under 5 years of age. There are data suggesting that the type 1 diabetes in very young children may have different genetic background. The aim of the study was to compare the frequencies of several genetic factors in two groups of children: diagnosed under 5 years of age and the group of older children.

Materials and Methods: The study group consisted of 357 children with type 1 diabetes aged from 0.33 to 18.2 (mean age 8.34, SD 4.61). In that group there were 99 children diagnosed under 5 years of age (mean age 2.68, SD 1.24). We analyzed the following markers: DRB1 alleles, CTLA-4 gene polymorphisms (+49A/G, CT60, MH30), six polymorphisms of Il-10 gene (592 C/A, 819 C/T, 1082 G/A, 2763 C/A, 2849 G/A, 3575 T/A), polymorphism 1085 C/T of PTPN22 gene, polymorphism -23 HphI of INS gene, polymorphism -1260 C/A of CYP27B1 gene, polymorphism rs 1544410 A/G of VDR gene and polymorphism rs 10774671 A/G of OAS1 gene. Two types of methods were used to analyze polymorphisms either PCR-RFLP or SnapShot minisequencing method.

Results: There were no statistically significant differences in frequencies of studied polymorphisms between children with diabetes diagnosed under 5 years of aged and those diagnosed over 5 years of age. For CTLA-4 gene polymorphism+49A/G p=0.92, OR=0.96, for CT60 p=0.60, OR=0.87, for MH30 p=0.67, OR=0.89, for haplotypes of Il-10 polymorphisms p=0.41, for polymorphism 1085 C/T of PTPN22 gene p=0.58, OR=1.14, for polymorphism -23 HphI of INS gene p=0.31, OR=0.75, for polymorphism -1260 C/A of CYP27B1 gene p=0.73, OR=1.08, polymorphism rs 1544410 A/G of VDR gene p=0.59, OR=1.12 and polymorphism rs 10774671 A/G of OAS1 gene p=0.29, OR=1.22.

Conclusion: The youngest children with type 1 diabetes do not differ genetically from children diagnosed at later age. It seems probable that the increasing incidence of type 1 diabetes reported recently in children under 5 years of age is due to some environmental factors that operate early in prenatal period or early infancy.

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0283

Identification of novel type 1 diabetes susceptibility loci in the Faroese population, a genetic isolate

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Background: Recent genome wide scans identify the MHC complex as the major susceptibility region for Type 1 diabetes. Non-MHC genes typically had lod scores below 3 and have minor contributions to Type 1 diabetes genetic susceptibility. As increasing numbers of Type 1 diabetic families are required for identification of non-MHC susceptibility genes the identification of these may be confounded by pooling of genetically heterogeneous populations. In genetic isolates patients with genetic diseases are likely to share extended haplotypes inherited from a few common founders. The Faroese Islands were populated at about 900 AD with a founder population of mainly Norwegians and British of a few thousand people. The size of this population remained stable at approximately 4000 inhabitants until around 1800. Since then the population has increased to its present level of 45.000 inhabitants. Thus this genetic isolate is almost ideal for genetic studies due to a probable strong founder effect. We hypothesize that

Faroese Type 1 diabetic patients share extended segments of DNA around disease genes allowing genome wide scans with fewer markers to identify novel susceptibility genes.
Aims: 1. To collect, clinically characterize and genotype with approximately 400 genetic markers the Faroese Type 1 diabetic families. 2. To identify novel Type 1 diabetes susceptibility genes.
Materials and Methods: We have succeeded in obtaining clinical information and DNA from 94% of the 110 Type 1 diabetic Faroese families, including 104 Type 1 diabetic probands and 294 relatives. We have performed a genome wide scan with 400 markers equal to a marker density of 10 cM. Based on genealogical evidence we have analyzed 21 families, of which 11 have a common founder and are unrelated in 6 generations.
Results: We have identified 6 chromosomal regions, with evidence for association to T1D, of which 5 were not previously reported. Using highly polymorphic markers in the HLA region we have shown that Faroese Type 1 diabetic patients do have genetic association with the MHC region and that this association does not differ between the 21 unrelated families and the remaining Type 1 diabetic families.
Conclusion: We have performed the first genome wide scan in the majority of Type 1 diabetic families from the Faroese genetic isolate. Five new genetic regions linked to Type 1 diabetes have been identified.
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0284

INS VNTR polymorphism in young onset diabetes subjects of Bangladesh

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Background and Aims: A remarkable heterogeneity in clinical and biochemical presentation creates a special problem in the characterization and classification of young diabetic patients in Bangladesh. In an attempt to characterize these patients we have studied INS VNTR polymorphism in young diabetic patients in relation to their insulin secretory capacity and autoantibody status.
Materials and Methods: A group of 372 young nonketotic diabetes mellitus (YDM) subjects (diabetes diagnosed under 30 years of age) and 332 healthy controls were studied. INS VNTR T/A polymorphism was analyzed by PCR RFLP using endonuclease *Hph*1 and serum C-peptide was measured by ELISA. Insulin secretory capacity (HOMAB) was calculated by Homeostatic Model Assessment. GAD antibody was determined by radioimmunoprecipitation method.
Results: Mean (±SD) BMI in the controls was 19.7±3.2 and YDM subjects 18.3±4.9 ($p<0.001$). Genotype frequencies of INS VNTR T/A polymorphism did not show any significant difference between Controls and YDM (homozygous wild 0.753 vs 0.712; heterozygous variant 0.229 vs 0.259 and homozygous variant 0.018 vs 0.030). HOMAB (%, median-range), of the YDM subjects [1.02 (0.11–2.11)] was significantly lower compared to the controls [1.94 (1.57–2.34), ($p<0.001$)]. In the controls HOMAB with wild genotype [1.94 (1.57–2.34)] did not show any difference with variant genotype [1.96 (1.68–2.32)], but in YDM group those with wild TT genotype [0.96 (0.11–2.17)] had significantly lower value compared to those with variant (TA and AA) genotype [0.1.13 (0.15–1.89), ($p=0.019$)]. GAD antibody was positive in 3.2% of the controls and 22.6% of YDM subjects. GAD antibody positive YDM cases had significantly lower HOMAB [0.67 (0.11–0.84)] compared to the negative cases [1.12 (0.11–2.17), ($p<0.001$)]. The proportion of wild genotype was significantly higher in antibody positive cases (83%) compared to negative cases (69%, $p<0.018$).
Conclusion: The association of INS VNTR wild TT genotype with GAD antibody positivity and lower insulin secretion suggest that young diabetic subjects in Bangladesh include a subgroup of typical type 1 diabetic patients with atypical clinical presentation and residual insulin secretory capacity.
The study was supported by grants from International Program in the Chemical Sciences (IPICS) and Diabetic Association of Bangladesh (DAB)

0285

HLA haplotypes and insulin gene VNTR polymorphism in relation to markers of beta cell autoimmunity in children from the general population

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Background and Aims: The emergence of beta-cell autoantibodies and their predictive value for type 1 diabetes (T1D) have been mostly studied in follow-up cohorts of children with a genetic predisposition for T1D. The present knowledge of the effects of HLA and insulin gene (*INS*) VNTR genotypes on the development of beta-cell autoimmunity may not be relevant in a genetically unselected population. Our aim was to retrieve information on early of beta-cell autoimmunity, and the association with HLA risk haplotypes and *INS* VNTR polymorphism in children from the general population.
Materials and Methods: The current study was part of a prospective population-based follow-up study of all infants born between 1st October 1997 and 1st October 1999 in Southeast Sweden (the ABIS study; All Babies in Southeast Sweden). We analysed autoantibodies to insulin (IAA), glutamic acid decarboxylase (GADA) and the tyrosine phosphatase like IA-2 (IA-2A) by radiobinding assays in sera taken from 714 children at 5–6 years of age. HLA genotyping was done using panels of sequence specific oligonucleotide probes, which were hybridized with amplified polymorphic regions of DQB1, DQA1 and DRB1 genes. A similar method was used for detection of polymorphism in the insulin gene at the −23 *HphI* A/T restriction site.
Results: We found that children from the general population with the risk associated haplotype DR4–DQ8 had an increased frequency of GADA above the 99th percentile at 5–6 years of age (p=0.005). The number of children positive for GADA and/or IAA above the 98th percentile tended to be lower in children with (DR15)–DQB1*0602 (p=0.056). The frequency of children positive for IAA, as well as for GADA or IA–2A, was similar in children with *INS*–23 A/A, A/T or T/T genotype.
Conclusion: The beta–cell autoantibody GADA is associated with the HLA DR4–DQ8 in our study population, but the impact of other disease associated risk genotypes on the development of autoantibodies seems to be small in a general population. These results suggest that the risk of beta-cell autoimmunity in a general population is not strongly associated with the risk genes of T1D other than the HLA DR4–DQ8 haplotype indicating that environmental factors are important determinants in the induction of beta-cell autoimmunity. The risk genes of T1D may rather determine the development from beta-cell autoimmunity to the destruction of beta-cells and clinical disease.
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0286

Progression to type 1 diabetes and autoantibody positivity in relation to HLA risk genotypes in children participating in the ABIS study

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Background and Aims: The incidence of childhood type 1 diabetes (T1D) has increased during the last decades, especially in children younger than 5 years of age. Autoantibodies against beta-cell antigens together with HLA risk genotypes can be used as predictive markers for development of T1D. The aim of this study was to describe the frequency of T1D and autoantibodies in children from the ABIS study and to investigate the relationship between autoantibodies and HLA risk and protective genotypes.
Materials and Methods: Autoantibodies against GAD (GADA) and IA-2 (IA-2A) were investigated at 1, 2.5 and 5 years of age in 2227 children, who participated in a prospective population-based follow-up study; the ABIS study (All Babies in Southeast Sweden). The frequency of children progressing to T1D was studied in all 17055 children from the ABIS study. GADA and IA-2A were determined with immunoprecipitation.
Results: *T1D:* By the age of 6–7 years 31 of total 17055 (0.18%) children in the ABIS study had developed T1D. 73% (16/22) had GADA and/or IA-2A before onset of the disease. HLA genotypes were analysed in 18 children and all had at least one susceptibility associated haplotype (p=0.0035 compared to autoantibody negative children) (table 1). *Permanent autoantibodies:* 16 children (0.7%) showed permanent GADA and/or IA-2A at both 2.5 and 5 years of age and 4 of them were also positive at 1 year of age. 9 of 15 children with permanent autoantibodies and studied for HLA genotypes were positive for HLA risk haplotypes (60%) (p=0.094 compared to autoantibody negative children), 3 of them together with a protective allele, and 6 had protective genotypes (40%). *Transient autoantibodies:* 374 children (17%) were positive for GADA and/or IA-2A at 1 and/or 2.5 years of age, but negative later. 29% of 363 children with transient autoantibodies and studied for HLA genotype had risk genotype without protective alleles, 17% had risk and protective alleles, and 41% had protective haplotypes, similar to figures seen in autoantibody negative children.
Conclusion: Most of the children with T1D developed GADA and/or IA-2A before diagnosis, indicating an ongoing autoimmune process. Permanent autoantibodies are rare in the ABIS population, while transient autoanti-

bodies occur more frequently. Induction of autoantibodies, especially transient occurrence, seems not to be controlled by HLA risk genotypes but rather by environmental factors. However, HLA risk genotypes play an important role for development of manifest T1D.

Table 1. HLA genotypes in relation to T1D and autoantibodies.

	Risk (S/S, S/N)	Protective (P/P, P/N)	Risk and protective (S/P)	Neutral (N/N)
Type 1 diabetes (18)	16 (89%)	0	2 (11%)	0
Permanent ab's (15)	6 (40%)	6 (40%)	3 (20%)	0
Transient ab's (363)	104 (29%)	151 (41%)	62 (17%)	46 (13%)
Negative ab's (357)	98 (28%)	126 (35%)	78 (22%)	55 (15%)

S=susceptible, N=neutral, P=protective haplotype

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0287

Promoter activity of the gene encoding repair enzyme protein carboxyl methyltransferase (*PCMT1*) is enhanced by exposure to INS-1e cells to cytokines

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Background and Aims: PCMT is an enzyme which recognises and repairs posttranslational isoaspartyl-formation in proteins. An increase in its expression is associated with a reduction in apoptosis in rat astroglial cells. The gene encoding this enzyme in humans, *PCMT1* is located on chromosome 6q24-25, in a region previously linked to type 1 diabetes (IDDM5). A variant in the promoter region of *PCMT1* (−509 C>G) which is in complete linkage disequilibrium with a functional variant in exon 5 (358G>A), was transmitted to 46% of the affected siblings in a sample of 248 Danish families with type 1 diabetes, and to 53% of the non-affected (p=0.099 for transmission to affected vs. non-affected). The aim of this study is to assess the response of *PCMT1* promoter activity to cytokine exposure in a murine insulin-producing cell line, as well as the potential functional consequences of the promoter variant previously described.
Materials and Methods: Seven promoter constructs were cloned upstream of the firefly luciferase (*luc*) reporter gene, comprising 1817–201 nucleotides upstream of *PCMT1* exon 1. All constructs were verified by sequencing and restriction enzyme cleavage. INS-1e cells (split numbers 65–89) were co-transfected with each reporter construct and renilla-luciferase (pRL-TK vector, internal control, Promega) using a cationic lysosome based method (SuperFect, Qiagen), in the absence or presence of IL-1, IFNγ or both. Luciferase activity was measured using an Orion micro-plate luminometer. Ratio of firefly/renilla luciferase activity was calculated for each construct and treatment. Five experiments were performed, with each condition in duplicate. Each treatment and promoter construct was compared with the rest (ANOVA, Tukey's post-hoc correction).
Results: Exposure to cytokines significantly increased promoter activity for all the constructs tested (firefly/renilla luciferase ratio 11.6±7.0 for unstimulated vs. 18.6±5.6 for IL1 vs. 16.4±5.3 for IFNγ and 20.1±6.8 for the cytokine mix, p≤0.003 for all cytokines vs. no treatment). The shorter construct showed significantly lower promoter activity (10.0±3.4, p≤0.05) than the rest (except for the 2 longest). There was no difference in the promoter activity of the constructs adjacent to the previously described variant (18.7±7.3 vs. 21.2±8.0, p=0.92).
Conclusion: *PCMT1* promoter activity is enhanced in INS1e cells by exposure to cytokines. The previously described variant in the *PCMT1* promoter region (−509 C>G) doesn't seem to have functional consequences on promoter activity.

PS 4

The grey zone between type 1 diabetes and type 2 diabetes – LADA

0288

Identification of two subtypes of adult-onset autoimmune diabetes (the NIRAD Study)

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Background and Aims: Autoimmune diabetes developing in adults is a clinically heterogeneous disorder, commonly identified by the pathogenetic marker glutamate decarbox-

ylase autoantibodies (GADA). In the present study we aimed to define heterogeneity of adult onset autoimmune diabetes based on characterization of GADA.

Materials and Methods: Patients enrolled in the NIRAD (Non Insulin Requiring Autoimmune Diabetes) Study, a nationwide survey aiming to assess prevalence and characteristics of adult onset autoimmune diabetes in Italy, have been screened for GADA and antibodies to IA-2 (IA-2A) and further characterized for GADA titre, antibodies to thyroid peroxidase (TPO) and HLA DRB1-DQB1 alleles.

Results: Of 4250 patients, enrolled between February 2001 and June 2004 in 83 diabetes centres in continental Italy, Sicily and Sardinia, 4.5% had either GADA and/or IA-2A. Of these, 189 (4.4%) had GADA, 39 (0.91%) had IA-2A and 37 (0.87%) had both. Analysis of GADA titres was independent from diabetes duration and showed a bimodal distribution which identified two subgroups of autoimmune patients, with high and low GADA titre, characterized by distinctive clinical and genetic features: compared with classical type 2 diabetes, high GADA patients showed significantly higher fasting glucose, HbA1c, prevalence of IA-2A, TPO antibodies, and frequency of DRB1*03-DQB1*0201 and lower age of diabetes onset, Body Mass Index, waist circumference, total cholesterol, triglycerides, uric acid and prevalence of metabolic syndrome (all $p<0.001$); patients with low GADA titre showed intermediate values, but still significantly different from type 2 diabetes for all these measures (all $p<0.03$ vs type 2), except for total cholesterol, triglycerides and prevalence of metabolic syndrome which were similar to type 2 diabetes. Frequency of DRB1*04-DQB1*0302 was comparable in all autoimmune patients and higher than in type 2 diabetes ($p<0.001$).

Conclusion: GADA titration allows a dissection of heterogeneity within adult onset autoimmune diabetes, with the identification of two subgroups of patients having distinctive clinical and genetic features.

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0289

Insulin resistance precipitates autoimmune diabetes in the adult (LADA) without affecting parameters of the immune process. Results from the Nord-Trøndelag Study

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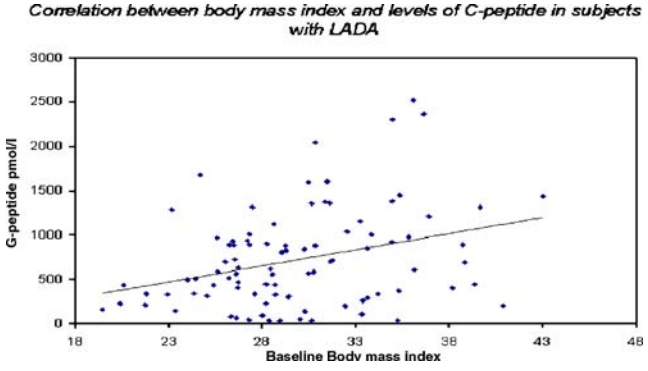
Background and Aims: The prevalence of latent autoimmune diabetes in the adult (LADA) is high, yet the risk factors for this type of diabetes are poorly characterized. The aim of this study was to investigate the influence of age, body mass index and physical activity on the 11-year incidence of LADA. For comparison, we also investigated these factors in relation to the incidence of Type 2 and Type 1 diabetes.

Materials and Methods: The study population consisted of 38,800 men and women of the Nord-Trndelag health study (HUNT). This is a prospective study where inhabitants of the county of Nord-Trndelag on two occasions (1984–86 and 1995–97) participated in a health examination and filled out a questionnaire on lifestyle factors. Patients classified as LADA had antibodies against glutamic acid decarboxylase (GAD) and were insulin independent at diagnosis.

Results: The results indicated that body mass index was strongly associated with LADA incidence. BMI $\geq$28 kg/m^2 at baseline was associated with a relative risk (RR) of 10.1 for LADA, 95% confidence interval (CI)=5.1–19.7, n=81. The association was similar for type 2 diabetes (RR=10.8, 95% CI=8.6–13.6, n=834) but was not found for type 1 diabetes (RR=0.55, 95% CI=0.12–2.55, n=24). Similarly, age was as important for the risk of LADA as for type 2 diabetes. Age $\geq$60 years compared to age <40 was associated with a relative risk of 5.6 (95% CI=2.4–13.4) for LADA and 6.8 (95% CI=5.1–9.1) for type 2 diabetes. In contrast, there was no clear association between age and type 1 diabetes (RR=0.86, 95%=CI 0.25–2.99). Subjects with high physical activity had a more than 50% reduced risk of LADA and Type 2 diabetes compared to sedentary subjects (RR=0.32, 95% CI=0.12–0.85 (LADA) and RR=0.49, 95% CI=0.37–0.66 (Type 2 diabetes)). These results persisted after adjustment for smoking (previously shown to be protective for LADA and Type 1 diabetes, Diabetologia 47, 1953, 2004). Baseline and follow-up BMI correlated strongly with fasting C-peptide in LADA and Type 2 diabetes but not with titres of anti-GAD.

Conclusion: We conclude that environmental and age-related insulin resistance precipitate diabetes in autoimmune-reactive individuals to the same extent as in Type 2 diabetes by increasing demands on beta-cell function but seemingly without affecting the immune process.



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0290

Action LADA, a multinational European project in latent autoimmune diabetes in adults (LADA)

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Background: LADA is defined by key features including adult age at diagnosis, the presence of diabetes associated autoantibodies and a delay in the need for insulin therapy. LADA represents about 5–10% of newly diagnosed non-insulin requiring diabetes patients. ACTION LADA aims to define the prevalence of LADA in 9 European countries including England, Northern Ireland, Denmark, Spain, Finland, Germany, France, Austria, and Italy. Entry criteria includes diagnosis of diabetes between 30 and 70 years, duration of less than 5 years, non-insulin requirement for the first 6 months of diagnosis. Accurate estimates of prevalence have important implications both for the correct classification of diabetes and potential for developing early intervention strategies.
Patients and Methods: We tested GADA from samples collected from participating centers in the Action LADA consortium using an IDS validated Radio-immuno-precipitation assay. GADA was standardized to IDS workshop and positivity defined as antibody concentration ≥25 WHO units.
Results: Of 5652 LADA samples identified according to the selection criteria, GADA were detected in 551 samples (10.6%). We analysed a single centre data from England according to GADA levels. Of 575 samples tested 416 were GAD negative, mean age 49 years (30-69). GADA was detected in 14.4% (83/575). Age at diagnosis was significantly lower in those who were GADA positive compared to GAD negative patients (43 Vs 49) ($p<0.0002$) however gender did not distinguish those with or without GADA antibodies. GADA positive patients were identified in 32% of the Insulin treated group in comparison to the non insulin treated group (7%) ($p<0.001$). Further analysis of these variables according to GADA levels showed a significant tendency for those with higher GADA levels to progress to insulin therapy.
Conclusion: We conclude that LADA is prevalent throughout Europe. In England GADA was associated with younger age at diagnosis and progression to insulin treatment. Preliminary evidence suggests heterogeneity within LADA according to GADA levels
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0291

Adult-onset autoimmune diabetes: identification of a new marker for the detection of anti-tyrosine phosphatase 2 (IA-2) antibodies (The NIRAD Study)

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Background and Aims: The presence of glutamic acid decarboxylase (GADA) and protein tyrosine phospatase IA-2 (IA-2A) in type 2 diabetic (T2DM) sera identifies those subjects that initially do not require insulin treatment but that are at high risk to develop future insulin dependency. Our aim was to identify, in Caucasian type 1 diabetes (T1DM) and adult-onset diabetes patients, the antigenic domains target of anti-IA-2 humoral autoimmunity, evaluating if age at disease diagnosis may influence the IA-2 immunoreactivity.
Materials and Methods: Adult-onset autoimmune diabetic patients were recruited from 4250 consecutive cases of T2DM enrolled in the NIRAD (Non Insulin Requiring Autoimmune Diabetes) study, a nationwide survey aiming

to assess prevalence and characteristics of adult onset autoimmune diabetes in Italy. The sera from n=88 T1DM (all at disease diagnosis T1DMa: n=44, mean age 7.1±2.8; T1DMb: n=44 mean age 29.6±5.1 yrs) and from n=125 adult-onset autoimmune diabetes patients, referred as NIRAD patients (NIRADa: n=31 mean age 44,2±13.8 within 6 months from diagnosis; NIRADb: n=94, mean age 52.6±12.6), with a disease duration of from 6 to 60 months) were analyzed. The reactivity of 5 IA-2 constructs [IA-2PTP(687–979), IA-2(761–964), IA-2(256–760), IA-2JM(609–631) e IA-2IC(605–979)] were tested using a radioimmunoprecipitation assay.

Results: T1DMa children had IA-2Abs mainly directed against the C-terminal portion of the protein, with significantly higher Ab frequencies for IA-2PTP(687–979) [T1DMa 57,5% vs T1DMb 15,9% ($p<0.0001$) and vs NIRADa 25,8% ($p=0,03$)] and for IA-2(761–964) [45% vs 19,4% ($p=0,042$)] with respect to adult TIDMb and NIRADa subjects. T1DMb and NIRADa sera reacted predominantly with the middle and juxtmembrane portion of the protein, with significantly higher IA-2JM(609–631) Ab frequencies with respect to T1DMa patients [T1DMb 27,3% and NIRADa 19,4% vs T1DMa 2,5%, $p=0.002$ and $p=0,04$ respectively]. No difference was found among the IA-2 construct reactivity relative to the different groups of adult diabetic patients (T1DMb, NIRADa e NIRADb). IA-2IC(605-979), actually considered the most sensitive construct for IA-2Ab detection, reacted with 65% of the T1DMa, 45.5% of the T1DMb and only with the 24.8% of NIRAD sera, detecting in this latter group of patients about 30% less positivity with respect to IA-2(256–760) (35,2%).

Conclusion: The results of this study indicate that a) the specificity of the IA-2 humoral immune response in diabetic patients is age-dependent b) the loss of IA-2 C-terminal immunoreactivity in adult diabetic patients results in a lower diagnostic sensitivity of the IA-2IC(605–979) fragment c) IA-2(256–760), an IA-2 construct lacking the C-terminal portion of the protein, may represent a new marker with high sensitivity for the study of the anti-IA-2 humoral immunoreactivity in adult-onset autoimmune diabetes.

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0292

The Protein Tyrosine Phosphatase Non receptor (PTPN22) is associated with high GAD antibody titre in adult-onset autoimmune diabetes (The NIRAD Study)

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Background and Aims: The Protein Tyrosine Phosphatase Non-receptor type 22 (PTPN22) gene encodes for lymphoid-specific phosphatase known as LYP, a powerful inhibitor of T-cell activation. Recently, many studies showed an association between the C1858T polymorphism (Arg620Trp) of the PTPN22 gene and type 1 diabetes (T1DM) and other autoimmune diseases. The aim of the present study was to evaluate whether the missense C1858T SNP of PTPN22 confers susceptibility to adult-onset autoimmune diabetes also referred to as Latent Autoimmune Diabetes in Adults (LADA) or Non Insulin Requiring Autoimmune Diabetes (NIRAD).

Materials and Methods: The study was performed in n=191 GADA positive patients recruited from 4250 consecutive cases of type 2 diabetes patients (T2DM) enrolled in the NIRAD (Non Insulin Requiring Autoimmune Diabetes) study. This NIRAD study is a nationwide survey aiming to assess prevalence and characteristics of adult-onset autoimmune diabetes in Italy. The bimodal distribution of GAD titres in antibody positive T2DM patients, allowed us to divide antibody positive (Ab+) patients into two subgroups, namely low (LAb+) n=94 and high (HAb+) n=97 GAD antibody titre positive patients. The analysis was also performed on n=382 T2DM patients (matched for sex, BMI age of recruitment and duration of disease), and defined as antibody-negative (Ab-), n=558 T1DM and n=235 controls. The C1858T SNP was genotyped using the fluorogenic 5′ nuclease assay application of the ABI PRISM 7900 HT Sequence Detection System. Statistical analysis was performed using SPSS statistical software, version 13. The genotype frequencies of the investigated SNP were in agreement with Hardy–Weinberg equilibrium. Genotype and allelic distributions of the C1858T polymorphism were compared using the x^2 test.

Results: The frequency of the 1858T carriers of the PTPN22 gene was significantly increased in High Ab +(16.2%) compared to Ab- (7%) ($p=0.01$), and even compared to Low Ab+(5.5%) ($p=0.02$). Low Ab+, Ab- patients and controls showed similar genotype and allelic distributions. Moreover, the PTPN22 1858T allele frequency was 8.8% in Low Ab+and 3.8% in Ab- ($p=0.01$). The 1858T phenotype confers an OR=2.6

Conclusion: In conclusion in GADA positive T2DM patients the C1858T polymorphism of PTPN22 gene is

associated with a high titre of GADA antibody rather than with the presence of autoimmunity "per se".
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0293

Plasma adiponectin in latent autoimmune diabetes of adults (LADA)

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Background and Aims: The role of adiponectin as an insulin-sensitizing protein in both subjects with type 2 diabetes and non-diabetics is widely accepted. Plasma adiponectin levels are known to show an inverse correlation with body mass index (BMI) in healthy individuals and to be decreased in patients with type 2 diabetes. In type 1 diabetes, plasma adiponectin concentrations were found to be higher than in healthy subjects, independently of BMI and diabetes control. Mechanisms for and the physiologic relevance of this finding are still unknown. The role of adiponectin in the Latent Autoimmune Diabetes of Adulthood (LADA), which shows characteristics of both type 1 (progressive autoimmune-mediated destruction of β-cells) and type 2 diabetes (insulin resistance), has not been investigated yet.
Materials and Methods: In a cross-sectional setting, we measured fasting adiponectin levels and explored their relationships to β-cell function and common metabolic parameters, as BMI and insulin resistance (estimated by homeostasis model assessment – HOMA–IR) in patients with LADA (n=33), with type 1 diabetes (n=41) and in healthy controls (n=72). Patients with LADA were identified by their age at diagnosis of diabetes (>40 years), a type 2 diabetes phenotype and the presence of GAD 65 and IA-2 antibodies.
Results: Patients with LADA had lower, compared to patients with type 1 diabetes, plasma adiponectin levels, adjusted for gender, age and BMI (p=0.006), and comparable levels to healthy controls. Inclusion of C-peptide levels in the model resulted in a highly significant inverse association of adiponectin levels with C-peptide in patients with type 1 diabetes (p=0.003) and LADA (p=0.002), but not in healthy controls (p=0.68). Even adjusted for C-peptide, patients with type 1 diabetes had higher adiponectin levels than patients with LADA (p=0.03). In patients with LADA, adiponectin was significantly associated with female sex (adjusted for age and BMI p=0.04) and negatively with BMI (adjusted for sex and age p=0.02) and HOMA–IR (adjusted p=0.03), whereas in patients with type 1 diabetes only with female sex (p=0.02).
Conclusion: Our results suggest that hypoinsulinemia is a major determinant of adiponectin levels in type 1 diabetes and LADA. However, in the latter, adiponectin levels are also affected by adiposity and inversely correlated with insulin resistance, suggesting a physiologic role similar to healthy subjects.

0294

Alteration of peripheral blood lymphocyte subsets in autoimmune diabetes of the adults (ADA) prior of insulin deficiency

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Background/Rational: About 10% of the patients diagnosed with T2DM, have detectable serum levels of autoantibodies against GAD65. These patients usually progress to insulin dependency within a few years and are classified as Latent Autoimmune Diabetes in Adults (LADA) or as suggested more recently Autoimmune Diabetes of the Adult (ADA). Specific changes in lymphocyte subsets, like a decrease in the frequency and function of peripheral blood NK cells as well as CD4+CD25+ regulatory T cells, have been observed in newly diagnosed T1DM and in pre-diabetic high-risk individuals. However, peripheral blood lymphocytes in ADA patients have been poorly investigated.
Aim: The aim of this study was to determine the frequency of major peripheral lymphocyte subsets in pre insulin-dependent ADA patients.
Methods: The frequency of peripheral blood lymphocyte subsets was determined in 44 ADA patients and 29 controls using four colour flow cytometry. All patients had autoantibodies against GAD65 in their blood but none of them were insulin-dependent. At the time of blood withdraw all patients were metabolically compensated.
Results: No differences were observed regarding the frequency of total T, CD4+, CD8+and B cells. A specific decrease of NK cell proportion was found in ADA patients. Moreover the frequency of total CD4+CD25+T cells was reduced in ADA patients.
Conclusions: These observations highlight a defect in the generation and frequency of NK and CD4+CD25+ lymphocytes in ADA patients. Recent evidences have

demonstrated a regulatory function of both these lymphocyte subsets. Thus, we suggest that these specific immunological alterations may contribute to the development of autoimmune diabetes by affecting important mechanisms of peripheral tolerance. Moreover, these data are in line with observations obtained in T1DM suggesting that similar immunological dysfunctions may contribute in both ADA and T1DM.

0295

The functional polymorphism (1858C/T) in the PTPN22 gene is associate with late autoimmune diabetes in adults – LADA.

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Background and Aims: The functional polymorphism (1858C/T) of gene encoding protein tyrosine phosphatase N22 (PTPN22) has been recently found to be associated with a higher risk of type 1 diabetes in several populations (including the Polish population). However, it is little known about the role of this gene polymorphism in patients with late autoimmune diabetes in adults (LADA). The aim of our study was to assess the association of the PTPN22 gene polymorphism 1858C/T and HLA DRB1, DQB1, DQA1 with the predisposition to LADA in the Polish population.
Materials and Methods: The study was performed in the group consisting of 175 individuals with classic clinical type 1 diabetes, 80 patients with LADA (age of diagnosis > 35 yrs., with anti-islet antibodies and low C peptide levels) and the control group comprising of 151 healthy non-related subjects. The GADA, IA2A and IAA antibodies were measured with RIA. HLA typing of DRB1, DQB1, DQA1 alleles was performed by PCR-SSP method. The presence of different variants of the PTPN22 gene polymorphisms was estimated using the DNA sequencing method.
Results: Patients with late onset autoimmune diabetes had a lower frequency of DRB1*03 (37.0% vs. 75.0%, $p<0.0001$), DRB1*04 (45.3% vs. 85.3%, $p<0.0001$) and DQB1*02 alleles (54.2% vs. 80.6%, $p=0.0007$) in comparison to those with type 1 diabetes, but still had the higher frequencies of these alleles in comparison to the controls. In contrast, the higher frequency of 1858 C/T polymorphism of PTPN22 gene have been shown in subjects with LADA in comparison to those with type 1 diabetes and healthy controls (1858T allele respectively: 26.8% vs. 17.4%, $p=0.013$, and vs. 13,2% $p=0,002$).
Conclusion: Our results shows, that similarly to type 1 diabetes, the HLA genes and non-HLA regions (PTPN22) play a role in the LADA genetic susceptibility, however different combinations of the risk alleles seems to influence the time of clinical onset of the disease.

0296

Association of *IRS-2 Gly1057Asp IRS-1 Ala513Pro* polymorphism with insulin resistance in patients with latent autoimmune diabetes in adults (LADA)

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Background and Aims: Presence of autoantibodies to GAD and/or ICA in patients diagnosed with type 2 diabetes (T2DM) is main diagnostic criteria of LADA. However results of some clinical study demonstrated that LADA comprises a heterogeneous population of diabetic patients, some of them may have insulin resistance.
The aim of the study was to evaluate the insulin sensitivity (IS) in patients with LADA during the first 5-years of disease and to investigate the association of *IRS-2 Gly1057Asp* and *IRS-1 Ala513Pro* polymorphism with LADA. It's important to understand the pathogenesis of this form of diabetes and for identification therapeutic approach.
Materials and Methods: We investigated 43 patients with latent autoimmune diabetes in adults (age 48,7±9,5 years, BMI 26,7±4,3 kg/m). The diagnosis of LADA was confirmed by the presence of autoantibodies (GAD65, ICA, IAA) in diabetes patients with initial diagnosis of T2DM, who didn't require insulin treatment for at least six months after the diagnosis. Control group consisted of 31 healthy volunteers (age 39,7±9,8 years old, BMI 22,9±3,6 kg/m.).IS was measured as Matsuda index (ISI) in 26 patients with LADA, by results of OGTT, and as CARO-index and HOMA-index. Data are given as means±SD. *IRS-2 Gly1057Asp* and *IRS-1 Ala513Pro* polymorphism was studied in 43 patients with LADA and 31 healthy controls.
Results: During OGTT patients with LADA had three types of insulin responses: hypersecretory, moderate and hyposecretory 53,7% patients with hypersecretory and moderate types had high insulin resistance (ISI 1,8±0,3 and 2,8±1,3). 46,3% LADA patients did not have stimulated insulin secretion and have high insulin sensitivity (ISI - 8,6±3,0). LADA was positively associated with the marker *Gly1057Asp* gene *IRS-2*. Distribution of alleles and genotypes frequencies of marker *Gly1057Asp* gene *IRS-2* was differently in LADA patient and control group. The presence of the *1057Asp* allele was significant higher in LADA patients (13,9% and 1,6%, respectively, $p<0,05$ OR=4,64 (1,46-14,6). The pres-

ence of the genotype *Asp/Gly* in codon 1057 of *IRS-2* in LADA patients was also significant higher than in control subjects (18,6% and 3,2%, respectively, p<0,05 OR=4,14 (1,01–16,8). (1,6% and 3,2%). In LADA group *1057Asp* allele was associated with lower BMI and waist-to-hip ratio and 8 from 10 LADA patients with *1057Asp* allele needs of insulin treatment during 5 years after the diagnosis. Patients with *1057Asp* allele had the same IS as patients with *Gly/Gly* genotype. We didn't find association between LADA and *IRS-1 Ala513Pro* polymorphism.

Conclusion: Our results demonstrated the association of *IRS-2 Gly1057Asp* polymorphism with latent autoimmune diabetes in adults. *1057Asp* allele may influence over early insulin dependency.

0297

Development of latent autoimmune diabetes of adulthood in patients, infected by hepatotropic viruses

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Background and Aims: To study the influence of hepatotropic viruses (HBV and HCV) on development of autoimmune processes to pancreatic beta-cells and it's secretory function in patients with diabetes mellitus (DM).

Materials and Methods: 173 patients with type 2 DM (41 male, 132 female, middle age 57.5±0.8 year, average diabetes duration 8.55±0.53 years) were surveyed. Patients were distributed into five groups: first group (n=7) – diabetic patients with HBV infection in replicative phase; second (n=68) – HBV infected in nonreplicative phase; third (n=23) – HCV infected in replicative phase; fourth (n=13) – HCV infected in nonreplicative phase; fifth (n=62) – noninfected type 2 diabetic patients. Markers of a viral hepatites B and C, glutamate decarboxilase autoantibodies (GADA), islet-cells cytoplasm autoantibodies (ICA) and basal C-peptide level were studied by immune-enzyme assay, viral DNA and RNA – by polymerase chain reaction.

Results: GADA was revealed more often in HBV and HCV infected type 2 DM patients in comparison with noninfected: in group 1–57.1%, p=0.007; in 2–36.7%, p=0.003; in 3–34.8%, p=0.003; in 4–53.9%, p=0.01 vs. group 5–9.7% (chi-square test). ICA in infected patients also met more often, than in noninfected: in group 1–28.6%, p=0.019; in 2–29.4%, p<0.001; in 3–17.4%, p=0.026, in 4–15.4%, p=0.15 vs group 5–1.6% (chi-square test). GADA and ICA revealing frequency was not depend on a virus replication. In 13.5% HBV- and HCV-infected patients both kinds of autoantibodies were determined. In noninfected patients simultaneously ICA and GADA wasn't revealed. In HBV and HCV infected, GADA and/or ICA-positive diabetic patients (n=18, patients with 0 pmol/l C-peptide level were excluded) the C-peptide median was essentially lower, than in GADA and/or ICA-negative diabetic patients (n=10) (0.84 vs 1.49 pmol/l respectively; p=0.027, Mann-Whitney U test). The survival analysis (Kaplan-Meier product limit method with use of Cox F-test) has been lead before an outcome (decrease C-peptide) depending on duration DM. It is revealed, that in HBV- and HCV-infected type 2 diabetic group a share of persons, which are not having C-peptide level decrease, was less, than in noninfected; the difference made from 8 up to 13% (F (158.66)=1.59, p=0.016).

Conclusion: In type 2 diabetic HBV and HCV infected patients autoantibodies to pancreatic beta-cells were found out statistically significantly more often, than in noninfected DM patients. In HBV and HCV infected patients with type 2 DM, with increase in duration of disease, decrease of insulin secretion faster progressed than in noninfected. One of the pathogenetic mechanisms of insulin secretion decrease can be autoimmune aggression directed to pancreatic beta-cells, initiated by HBV- and HCV-infection. In 36% of HBV- and HCV-infected diabetic patients were found out both autoantibodies to beta-cells and down level of insulin secretion that testifies to development in them of latent autoimmune diabetes of adulthood.

PS 5

The spectrum of diabetic disorders

0298

Permanent neonatal diabetes could be much more frequent than estimated: Slovak incidence and successful transfer to sulphonylureas

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Background and Aims: Permanent neonatal diabetes (PND) has been recently intensively investigated across Europe. Nonetheless, the information on frequency of PND cases has been based on non-population based collections only. Thus the aim of this study was to identify the incidence for PND in Slovakia and to transfer these patients to sulphonylureas (SU).
Materials and Methods: We have searched for PND patients in the Slovak National Diabetes Registry (SNDR), which includes all children with diabetes since 1981. In the insulin treated patiens who matched the clinical criteria for PND, the KCNJ11 gene was sequenced and the KCNJ11 mutation carriers were invited for transfer to SU.
Results: Based on information from SNDR, eight patients with diabetes onset under 6 months were identified. With a mean of 71,805 live births per year since 1981, the PND incidence in Slovakia is approximately one case in 215,000 live births. One of the 8 PND identified had died and one has the Wolcott-Rallison syndrome. In four of the remaining six patients, 3 different KCNJ11 mutations were found (i.e. R201H, and two novel mutations: H46Y and L164P). Three patients (two R201H and H46Y carriers) were transferred from insulin to SU. Before therapy change, the subjects had an insulin requirement of 0.88 U/kg/day (0.66–1.00), HbA1c 10.0% (9.3–11.0) and C-peptide concentrations 0.06 nmol/ml (0.04–0.11). All subject responded well to SU and insulin was stopped. After 1 month off insulin, the HbA1c decreased to 6.7% (5.9–7.3) and the C-peptide concentrations increased to 0.73 nmol/ml (0.61–0.79). Insulin and C-peptide secretion response to oral glucose (in OGTT) was higher than to intravenouse glucose. The L164P carrier suffers from active chronic hepatitis C, and so far has not been suitable for the treatment change.
Conclusion: The PND frequency in Slovakia is approximately one case to 215,000 live births, which is much higher than previous international estimations (1 in 800,000 live births). We have identified 4 KCNJ11 mutation cariers and 3 of them sucessfully tranferred to SU, dramatically improving their diabetes control and quality of life.
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0299

Kir6.2 mutation search in patients with PND from a Polish population. Successful transfer to sulphonylurea therapy in children and adult subjects

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Introduction: Activating mutations in the *KCJN11* gene encoding Kir6.2 have been described in patients with permanent neonatal diabetes (PND). **Aim:** To examine the contribution of *KCJN11* mutations to PND in patients from Poland.
Material and Methods: To dissect the genetic background of PND in Poland, the Nationwide Registry was established. So far, 32 subjects were included, all diagnosed below 6 months (range 1–26 weeks). This constitutes one of the world's largest national PND collections. Until now, we sequenced the *KCJN11* gene in 25 insulin treated, diabetic patients (age at exam: 1–50 years).
Results: We identified eleven patients with heterozygous missense diabetes causing mutations. Five subjects carried the R201H mutation, three had the V59M substitution, one patient was a carrier of the R50Q, the H46L, and the K170N mutations, respectively. There was evidence of clinical heterogeneity: the age of diagnosis varied from 2 to 26 weeks; and insulin requirements varied from 0.25 to 1 U/kg. During euglycemic hyperinsulinemic clamp in three adult R201H mutation carriers, we found various degrees of insulin resistance. So far, ten patients, seven children and three adults, (range 5–50 years) were transferred to sulphonylurea therapy (SU). In five cases with R201H mutation we used controlled-release glipizide GITS (gastrointestinal therapeutic system). One carrier of R50Q mutation was well controlled on 1 mg of glimepiride. In four children with evidence of developmental delay (V59M and H46L mutations) glibenclamide was successfully used. HbA1c improved in all patients. To our knowledge, a 50 year old man transferred by us to glipizide is the oldest human with the Kir6.2 mutation in whom SU was used. We were not able to fully transfer a 20 year old carrier of K170N mutation who is currently on combined glibenclamide/insulin regimen.
Conclusions: We summarize that Kir6.2 mutations are a common cause of PND in a Polish population. The results showed evidence of clinical heterogeneity among Kir6.2 mutation carriers. We report successful transfer of paediatric and adult patients from insulin to various SU (sustained-release glipizide, glimepiride, and glibenclamide).
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0300

Catalytic instability of the glucokinase MODY-2 mutants V62M and G72R in pancreatic beta cells

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Background and Aims: The glucokinase (GK) mutations V62M and G72R demonstrate mildly activating kinetics and are thermally stable compared with wildtype GK but paradoxically cause Maturity Onset Diabetes of the Young Type 2 (MODY-2). Lack of responsiveness to a pharmacological activator and the liver regulatory protein GKRP has been reported. However, neither abnormality can explain the defect in insulin secretion in MODY-2. Therefore, the aim of the present study was to use a cell biology approach to determine the mechanism by which these mutants compromise GK function in pancreatic beta-cells.
Materials and Methods: To investigate cellular stability, expression and subcellular location of the mutant GK, stable pancreatic beta-cell MIN6 cell lines expressing GFP-tagged wildtype, V62M and G72R GK were generated.
Results: As proteosomal inhibition revealed increased turnover of GFP-tagged GK in the stable cell lines, kinetic and cellular analysis was performed during proteosomal suppression. Kinetic analysis of the stable cell lines confirmed the mildly activating kinetics of the mutant enzymes within the pancreatic beta cell. GFP-GKwildtype demonstrated similar sigmoidal kinetics to untransfected MIN6 cells ($S_{0.5}$=9.0±1.0 mM vs 9.1±1.3 mM), whilst GFP-V62M and GFP-G72R demonstrated a decreased $S_{0.5}$ for glucose (5.2±0.4 mM and 4.6±1.0 mM, respectively). GFP-GKwildtype demonstrated activation by a pharmacological GK activator ($S_{0.5}$=1.9±0.2 mM) whereas GFP-V62M and GFP-G72R did not respond to the GK activator. Similarly, GFP-GKwildtype demonstrated appropriate interaction with liver GKRP whilst neither mutant protein demonstrated interaction with GKRP, either in hepatocytes or following adenoviral expression in the stable beta-cell lines. Cellular stability analysis revealed a decreased GK activity/immunoreactivity and GK activity/mRNA ratio for GFP-V62M and GFP-G72R compared to GFP-GKwildtype (2–4fold), both in the absence and presence of proteosomal suppression, indicating catalytic instability. Protein stability, determined by the immunoreactivity/mRNA ratio, was similar for GFP-GKwildtype, GFP-V62M and GFP-G72R.
Conclusion: The GK mutants V62M and G72R demonstrate cellular catalytic instability in pancreatic beta-cells and are not responsive to a pharmacological GK activator or liver GKRP. The catalytic instability of V62M and G72R may explain the clinical MODY-2 phenotype.
Supported by Diabetes UK

0301

The −30G>A polymorphism in the glucokinase gene predicts the effect of a lifestyle intervention on insulin secretion in a population at risk for type 2 diabetes

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Background and Aims: Glucokinase is considered to play a key role in glucose sensing and regulation of insulin secretion. The impact of the −30 G>A polymorphism in the β-cell specific promoter region of the glucokinase gene on insulin secretion has been investigated in cross-sectional studies yielding inconsistent results. We investigated the long-term effect of the polymorphism on insulin secretion in the setting of a lifestyle intervention program to prevent type 2 diabetes.
Materials and Methods: The effect of the −30G>A polymorphism on insulin secretory function in our population was determined in 116 non–diabetic subjects by means of a hyperglycemic clamp using different secretagogues. During a lifestyle intervention with diet and increase in physical activity we investigated whether this polymorphism predicted the change in glucose-stimulated insulin secretion as assessed by first phase insulin secretion and 30 minute C-peptide plasma levels during an oral glucose tolerance test.
Results: The −30A allele was associated with lower first (p=0.03) and second phase (p=0.02) of glucose-stimulated insulin secretion, and with lower second phase of GLP-1 stimulated insulin secretion (p=0.007) independently of age, gender and insulin sensitivity. During the lifestyle intervention in subjects with impaired glucose tolerance, there was an increase in insulin secretory function in homozygote carriers of the −30G allele, while there was a decrease in carriers of the −30A allele, resulting in a significant difference in the change in insulin secretion between genotypes (first phase: p=0.009; 30 min C-peptide: p=0.01). In subjects with normal glucose tolerance no difference in the change in insulin secretion was found between genotype groups.
Conclusion: In German population at risk for developing type 2 diabetes, carriers of the −30A allele in the glucokinase gene have lower insulin secretory capacity than G/G homozygotes. Intervention for preventing diabetes in these subjects should

be carried out as early as they maintain normal glucose tolerance, because, once impairment of insulin secretion is established, it continues to decline, even when a lifestyle modification program is implemented.

0302

The impact of HNF1β (R276X) mutation on insulin secretion and morphology of kidney

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Background and Aims: HNF1β is a transcription factor that expresses in pancreatic β cells and has an important role in the development and maintenance of islet cell function. A definite evidence for its significance in islet function is phenotypic expression of MODY5 patients with the HNF1β gene mutation, but the mechanism for the development of diabetes and polycystic kidney disease remains unknown. Here we report observations of a case with HNF1β (R276X) mutation, a second case in the world. The aim of this study is to examine the clinical phenotype, histology of the kidney and affectedness to insulin secretion.
Methods: Genomic DNA was extracted from peripheral blood sample, followed by direct sequencing. Imaging studies of kidneys, consisting of ultrasonography and computed tomography, and renal biopsy specimens were reviewed by a nephrologists and a renal pathologist. To clarify the physiological relevance of HNF1β mutation *in vitro*, we monitored insulin secretion indirectly by measuring human C-peptide secretion by transfecting HNF1β mutant cDNA with human preproinsulin cDNA into insulin secreting cell lines.
Results: The patient is a 31 year-old male who was diagnosed as an insulin-requiring diabetes at age 27. It was unlikely that he had type 1 diabetes because basal insulin secretion is relatively low but his urine C-peptide excretion still remained detectable by 36.1 μg/day, islet-associated autoantibodies including anti-GAD, anti-IA2 and anti-ICA antibodies were all negative, and also he had no susceptible HLA allele to type 1 diabetes. In the family history, his father had developed renal failure with mild diabetes but had already past. By clinical examination, size of his left kidney was small (85 mm in the height) accompanied by cortical atrophy, and right kidney had been extensively occupied by multiple cysts. The creatinine clearance was reduced as low as 54 ml/min though he had no detectable proteinuria, suggesting that the renal damage is not based on common diabetic nephropathy. Electron microscopic examination of left kidney whose sample was obtained by needle biopsy revealed presence of focally enlarged glomeruli, but no evidence of typical diabetic nephropathy. Direct sequence analysis of genomic DNA has revealed that his one allele of HNF1β has C to T point mutation that leads to truncated protein in exon 4. In order to assess its functional relevance, we assayed C-peptide concentration by transfecting human preproinsulin gene to hamster insulinoma (HIT) cell line. When simultaneously transfecting the mutant HNF1β gene with insulin gene, excess expression of R276X-HNF1β gene did not change concentration of human C-peptide in culture media, suggesting that the mutation has little influence on basal insulin secretion as a dominant negative manner.
Conclusion: We have identified HNF1β mutation HNF1β (R276X) with small kidney and multiple renal cysts for the second time. This is the first report that showing imaging studies of kidneys of HNF1β mutation. Additional studies are needed to further clarify the relevance between HNF1β mutation and damped insulin secretion.
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0303

Familial persistent hyperinsulinaemic hypoglycaemia of infancy due to a novel glucokinase *(GCK)* activating mutation G68V

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Background & Aims: Persistent hyperinsulinaemic hypoglycaemia of infancy (PHHI) is a rare heterogenous disorder. Heterozygous activating mutations in the glucokinase (*GCK*) gene have been described in a small number (n=6) of cases. Patients with *GCK* activating mutations have regulated insulin secretion and can usually be treated with diazoxide. We identified a 7 year old female proband with a history of hypoglycaemia from infancy. The proband's mother, maternal-grandmother and 2 siblings were also affected. Clinical investigations in the proband demonstrated that hypoglycaemia (fpg 2.3–3.5 mmol/L with corresponding insulin values of 50–110 pmol/L) was the only abnormal finding. The patient is successfully treated with diazoxide and a biopsy revealed that pancreatic histology is normal. Given this clinical phenotype we hypothesised that the proband had an activating mutation in the *GCK* gene.

Methods: The entire coding region and exon–intron boundaries of the human islet *GCK* gene were sequenced in the proband and her family. Functional analysis of purified wild-type (WT) and mutant G68V recombinant glutathionyl S-transferase fusion proteins were performed spectrophotometrically using an NADP coupled assay.
Results: We have identified a novel *GCK* mutation (exon 2, GGC>GTC, G68V) which co-segregates in the family with hypoglycaemia. This mutation is not present in 200 normal chromosomes. Structural modelling localised the mutated residue to the allosteric activator site. Kinetic analysis revealed that G68V-GCK is 22 times as active as WT- GCK with an increased affinity for glucose (glucose $S_{0.5}$ 1.70±0.18 for G68V-GCK vs 7.25±0.34 mmol/L for WT-GCK), a decreased ATP K_m (0.27±0.06 for G68V-GCK vs 0.53±0.03 mmol/L for WT-GCK), the maximal specific activity (K_{cat}) and Hill number were unchanged. Mathematical modelling predicted a threshold for glucose stimulated insulin (GSIR) release of 1.45 mmol/L.
Conclusion: We have identified a novel activating mutation in the *GCK* gene in a family with PHHI. Our results increase the number of reported cases of GCK-PHHI and add to our knowledge of the GCK activator site which is an important target for therapeutic agents.
Supported by Diabetes UK

0304

The role of a family history of type 2 diabetes in patients with type 1 diabetes (the FinnDiane Study)

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Background and Aims: Non-diabetic offspring of type 2 diabetic patients (T2D) are insulin resistant and show components of the metabolic syndrome. We have recently reported a 39% prevalence of the metabolic syndrome in Finnish type 1 diabetic patients. In the present study, the aim was to study the association between family history of T2D and clinical characteristics, as well as presence of diabetic late complications in patients with type 1 diabetes.
Materials and Methods: Of 1860 type 1 diabetic patients from the cross-sectional Finnish Diabetic Nephropathy (FinnDiane) Study, 620 with and 1240 without a family history of T2D were matched for age and sex. Family history of T2D was based on the information provided by the type 1 diabetic proband using a standardized questionnaire (sensitivity 89%, specificity 98%).
Results: The mean age of the probands was 43.5±0.2 and 50% were male. The table below shows characteristics of patients with (FH T2D+) and without (FH T2D-) a family history of T2D. BMI=body-mass index, eGDR=estimated glucose disposal rate.

	FH T2D+ *n*=620	**FH T2D-** *n*=1240	***P* value**
Age at onset of diabetes (yrs)	17.2±0.4	16.1±0.3	0.008
BMI (kg/m^2)	25.7±0.2	25.0±0.1	<0.001
Wiast cicumference (cm)	88.0±0.5	86.1±0.3	0.002
HbA_{1c} (%)	8.4±0.1	8.3±0.1	0.020
eGDR ($mg*kg^{-1}*min^{-1}$)	5.8±0.1	6.1±0.1	0.023
Insulin dose (IU/kg)	0.68±0.01	0.65±0.01	0.005
Triglycerides (mmol/l)	1.36±0.04	1.20±0.02	<0.001
HDL Cholesterol (mmol/l)	1.37±0.02	1.38±0.01	0.478
Systolic blood pressure (mmHg)	138±1	137±1	0.195
Diastolic blood pressure (mmHg)	80±1	80±1	0.570

In a logistic regression analysis, an independent association with family history of T2D was found for age at onset of diabetes, BMI, insulin dose, and triglycerides. Family history of T2D was associated with the metabolic syndrome (NCEP criteria; 44% vs. 38%, p=0.014, IDF definition; 43% vs. 38%, p=0.031), but not with coronary heart disease (9.9% vs. 7.4%), cardiovascular hard end-points (13% vs. 12%), diabetic nephropathy (25% vs. 25%), retinal laser treatment (44% vs. 44%), or antihypertensive medication (49% vs. 49%). Maternal history of T2D was associated with larger waist circumference, lower eGDR, higher triglycerides, HbA_{1c}, insulin dose, BMI, and later onset of diabetes, as well as coronary heart disease, cardiovascular hard end-points, and the metabolic syndrome (NCEP and IDF). Paternal history of T2D was associated with higher triglycerides, insulin dose, and BMI.
Conclusion: A family history of T2D is associated with some, but not all, components of the metabolic syndrome, and also with a later onset of diabetes. In these still rather young type 1 diabetic patients, a family history of T2D was not associated with diabetic late complications.

0305

HLA-DQB1 genotypes predict autoimmune diabetes development and β cell failure in adult (40–59 years old) diabetic patients

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Background and Aims: Common allelic variants at the HLA class II loci account for the major genetic risk for type 1 diabetes among children and young adults. However, HLA predisposition to autoimmune diabetes development among adult diabetic patients is not well established. We assessed HLA-DQB1 genotypes, islet antibodies (GADA) and fasting plasma C-peptide (F–P–C-peptide) among 1339 Swedish adult (40–59 years old) diabetic patients from a diabetes registry in Southern Sweden. Our aim was to relate HLA–DQB1 genotypes to GADA and β-cell function (F–P–C-peptide) in adult diabetic patients.

Materials and Methods: Age at onset of diabetes was between 40–59 years in registered patients. F–P–C-peptide concentration was measured in duplicate by a radioimmunoassay: normal range was 0.25–0.75 nmol/l. GADA were measured by radioimmunoprecipitation method: cut-off limit for positivity was 5 RU. HLA-DQB1 genotyping was performed using hybridisation assay.

Results: Among the 1339 patients, risk HLA-DQB1 genotypes were found in 283 (21%), GADA in 170 (13%) and low F–P–C-peptide (<0.25 nmol/l) in 172 (13%). Patients with GADA had significantly ($p<0.0001$) lower median BMI (25 vs. 29 kg/m^2) and F–P–C-peptide (0.3 vs. 0.9 nmol/l) than those without GADA. Compared with GADA−, GADA +was associated with female gender (49% vs. 35%; $p=0.0001$) and insulin treatment (60% vs. 23%; $p<0.0001$). HLA-DQB1 *0302/X were increased in diabetic patients compared with non-diabetic controls (10% vs. 4.2%; $p=0.0049$). After stratification for GADA presence, compared with GADA−, GADA+had a fivefold increased risk of having HLA-DQB1 *02/*0302 (27% vs. 6.7%; $p<0.0001$), significantly ($p<0.0001$) lower (<0.25 nmol/l) F–P–C-peptide (6.6% vs. 48%) and lower (<25 kg/m^2) BMI (20% vs. 50%). In multinominal regression analysis, HLA-DQB1 *02/*0302, low F–P–C-peptide (<0.25 nmol/l), low BMI (<25 kg/m^2) and female gender remained independently significant in association with GADA. Evaluation of F–P–C-peptide concentrations separately in GADA+and GADA− groups related to diabetes duration and HLA-DQB1 genotypes showed that, low F–P–C-peptide significantly (<0.0001) correlated with a long duration of diabetes, both in GADA+($r=-0.445$) and GADA− ($r=-0.179$). Moreover, amongst GADA+, low F–P–C-peptide concentrations were significantly associated with HLA-DQB1 *02/*0302 ($p=0.039$), whereas amongst GADA−, low F–P–C-peptide concentrations were significantly associated with HLA-DQB1 *0302/X ($p=0.001$) and *0302/*0604 ($p=0.033$). After stratification of F–P–C-peptide concentrations into (<0.25 nmol/l) and (>0.25 nmol/l), multinominal regression showed that GADA+($p<0.0001$), HLA-DQB1 *0302/X ($p=0.018$), *0302/*0604 ($p=0.006$), low (<25 kg/m^2) BMI ($p<0.0001$) and a long duration of diabetes ($p<0.0001$) were independently associated with low (<0.25 nmol/l) F–P–C-peptide concentrations.

Conclusions: Although decrement of F–P–C-peptide concentrations is associated with a long duration of diabetes, risk HLA-DQB1 genotypes independently predict autoimmune diabetes development and β-cell failure in 40–59 years old diabetic patients: *02/*0302 is associated with GADA+, but GADA+independently of HLA-DQB1 risk, contributes to β-cell failure, whereas among GADA−, HLA-DQB1 *0302/X and *0302/*0604 contribute to β-cell failure. Hence, our study suggests that risk HLA-DQB1 genotypes contribute to β-cell failure in patients without GADA.

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0306

Epitope specificity of autoantibodies directed to the N-terminus of glutamate decarboxylase (GAD65) correlate with beta cell functional reserve in patients with ketosis prone diabetes mellitus

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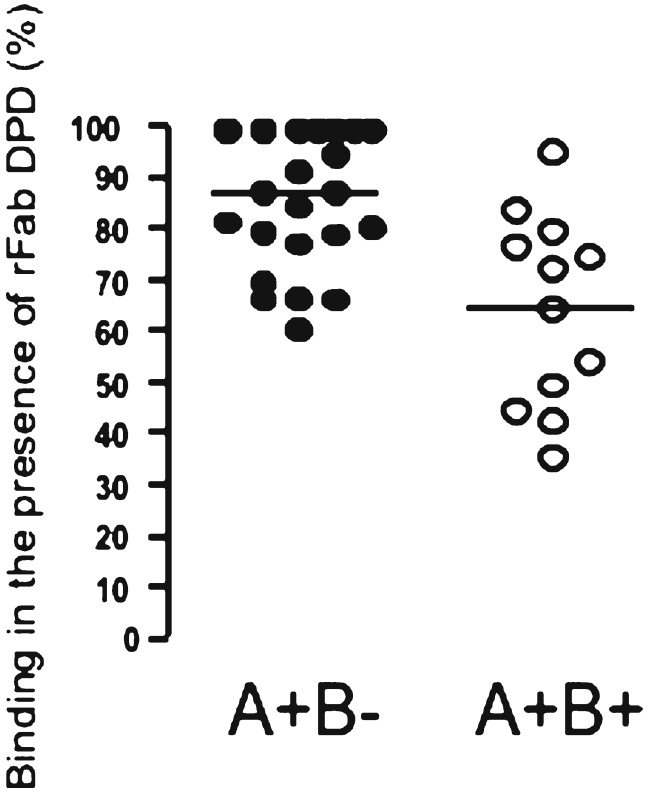
Background and Aims: Diabetic ketoacidosis (DKA) has traditionally been viewed as a complication of type 1 diabetes, but several recent studies have shown that patients presenting with DKA are heterogeneous in regard to the type of diabetes. We have prospectively characterized these heterogeneous forms of ketosis-prone diabetes mellitus (KPDM) into four subgroups, based on the presence or absence of beta cell autoimmunity (A+or A−) and beta cell functional reserve (B +or B−). The A+B− KPDM subgroup comprises patients with classic, autoimmune type 1 diabetes (complete beta cell loss), whereas the A+B+ KPDM subgroup, despite possessing the same autoantibodies as A+B− patients, have only partial beta cell loss and a distinct clinical phenotype. We hypothesized that the autoimmune reaction differs between these two KPDM subgroups. We analyzed the epitope specificity of autoantibodies directed against the 65 kDa isoform of glutamic acid decarboxylase (GAD65) as a reflection of the underlying islet cell autoimmunity.

Material and Methods: We analyzed the sera of GAD65Ab-positive KPDM patients (n=36) for their epitope recognition using recombinant Fabs specific to six different GAD65 epitopes. Binding to the different epitopes was correlated with measures of beta cell function (HOMA%B, Fasting C-peptide (FCP)), age, gender and other clinical parameters.
Results: The sera's binding to a GAD65 N-terminal epitope correlated with longitudinally assessed beta cell functional reserve. Binding to the N-terminal epitope correlated with FCP (R=0.56, p=0.0008) at the initial sampling and with both HOMA%B and FCP 12 months later (R=0.4, p=0.03, R=0.54, p=0.007, respectively). In the two phenotypic subgroups of GAD65Ab-positive patients, binding to the N-terminal epitope was significantly stronger in A+B+(median binding 31%) than in A+B− patients (median binding 12%) (p=0.001).
Conclusion: Binding to a N-terminal epitope of GAD65 is correlated with preserved beta cell functional reserve in GAD65Ab-positive patients with KPDM, and therefore associated with the A+B+phenotype. Whether GAD65Ab specific to the N-terminal epitope can be used as markers for a slower or more latent form of beta cell destruction or may even be involved in this more benign clinical course, remains to be investigated.



Supported by American Diabetes Association

0307

Autoimmunity and prevention of type 2 diabetes: the Diabetes Prevention Program (DPP)

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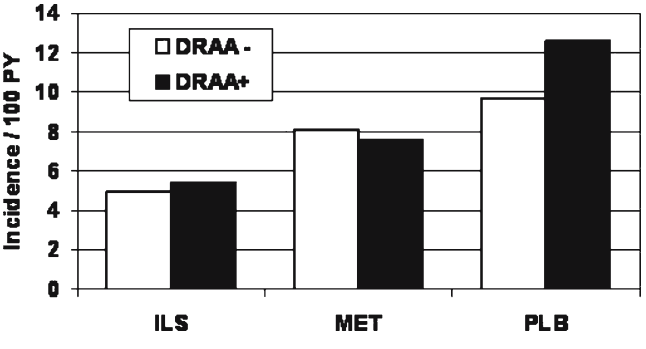
Limited data exist on the prevalence and predictive role of diabetes-related autoantibodies (DRAA) among adults at risk for DM. Moreover, it is unknown whether preventive interventions are equally effective in subjects with and without DRAA. The DPP measured baseline GAD65 and IA-2 antibodies in 3,050 IGT subjects aged 25–84, randomized to intensive lifestyle (ILS; N=1015), metformin (MET; N=1013) and placebo (PLB; N=1022) and followed 3.2 years. DRAA positive (DRAA+) was either GAD65 index ≥0.085 or IA-2 index ≥0.017. Cox models assessed the effect of DRAA on DM risk, accounting for the potential interaction with treatment assignment, and adjusted for age, sex, race/ethnicity, baseline BMI, HbA1c, insulin resistance and beta-cell function. A total of 367 subjects (12%) had DRAA+: 9.5% of non-Hispanic whites, 9.7% of Hispanics, 16.0% of African–Americans, 14.1% of Asians and 29.6% of American Indians. Race/ethnic differences were mostly due to IA-2. Crude DM incidence by DRAA and DPP intervention is shown in the figure. Overall, DRAA did not predict DM [Hazard ratio, HR=1.18 (95%CI=0.96–1.46)]. However, DRAA+persons in the PLB group had 40% higher DM risk (CI=3–91%) than DRAA negative. The increased risk in the PLB group was largely conveyed by IA-2 alone [HR=1.50 (1.0–2.4)]. The effects of ILS or MET (vs. PLB) on DM prevention were similar regardless of DRAA (p=0.3 for interaction). In conclusion, among people with IGT, DRAA predict faster progression to DM in untreated persons, and ILS and MET are equally effective in subjects with and without autoimmunity.



PS 6

Incidence and prevalence of type 2 diabetes in different ethnic populations

0308

Incidence of type 2 diabetes in Pima Indian and Mexican populations

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Background and Aim: Pima Indians from Arizona have the greatest incidence of type 2 diabetes in the world, while in Mexico type 2 diabetes prevalence has increased over the last decades. The aim of this study was to compare the adjusted incidence of type 2 diabetes in two longitudinal studies from these populations with differences in genetic susceptibility and in the prevalence of risk factors.
Methods: A population based follow up study began in 1989 in low income neighborhoods of Mexico City with 1460 subjects (592 men and 868 women) free of diabetes and aged 35 to 64 years at baseline. Follow up visits, including fasting and 2 hours plasma glucose, were performed on average every 3 years up to 2000. A study in Pima Indians started in 1965 with biannual follow up visits up to 2004, similar to the ones in the Mexico City cohort. From this study we included 1,466 subjects (615 men and 851 women) with their first non diabetic examination between ages 35 to 65 years. Incidence rate (IR) was calculated as the number of persons who developed diabetes divided by the person-years of follow-up and expressed per 1,000 person-years and standardized to the 1998 World population aged 35 to 65 years. The risk period extended up to the examination at which diabetes was diagnosed or to the date of the last examination within the study period in each cohort. Diabetes was diagnosed by the WHO criteria (fasting plasma glucose>7.8 mmol/l or 2 hour post load plasma glucose>11.1 mmol/l).
Results: At baseline, Pima Indians had worse risk profile than Mexican participants, especially for mean BMI (33.3 vs 28.0 kg/m2), fasting plasma glucose (97.6 vs 84.7 mg/dL) and 2 hour post load plasma glucose (120.1 vs. 104.72 mg/dL). During a median follow-up of 5.6 years (range 1.0 to 9.5 years), 161 Mexican subjects developed diabetes. Age-adjusted IR were 18.5 cases/1000 pyrs (95% CI 13.9–23.13) in men and 19.2 (95% CI 15.2–23.1) in women. For Pima Indians, during a median follow-up of 12.6 years (range 0.05–39.2 years) 607 of them developed diabetes. Age-adjusted IR were 41.6 cases/1000 pyrs (95% CI 36.0–47.3) in men and 53.6 (95% CI 48.2–58.9) in women. The sex-adjusted IR stratified by age categories are presented in table 1. Given the differences on risk factor at baseline, we selected a subset from the Mexican cohort with high risk to develop diabetes defined as fasting plasma glucose>100 mg/dL at baseline. The age and sex adjusted IR on this high risk group of Mexicans was 41.3 cases/1000 pyrs (95% CI 34.2–48.4) similar to the IR in the general Pima Indian population.

Sex-adjusted IR (per 1,000/year) of diabetes in Mexicans and Pima Indians by age category

	Mexicans		Pima Indians	
Age (years)	IR	95% CI	IR	95% CI
35–44	14.3	9.8–18.9	41.6	36.0–47.3
45–54	24.7	19.1–30.4	54.4	47.4–61.3
55–64	19.4	13.7–25.2	49.5	40.1–58.9
Age–sex-adjusted	18.9	15.8–21.8	47.6	62.9–88.8

Conclusions: The IR of diabetes in Pima Indians is more than 2 times higher than that in Mexican subjects. However this difference does not hold once we select a high risk subset of the Mexican population, suggesting that the original difference is partially explained by differences in baseline risk factors.

0309

Increased prevalence of diabetes in an industrial area of southern Poland

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Background and Aims: Prevalence of diabetes is growing up and global data shows the increasing burden. No realistic data on epidemiology of diabetes from Poland, for the last 20 years, is available. Aim: Estimation of the prevalence of diabetes and cardiovascular risk factors in industrial area of Southern Poland in adult population.
Materials and Methods: We randomly selected and invited for screening the group of 782 subjects from the voters' list consisting of 23442 inhabitants of suburb Halemba, town Ruda Slaska. The attendance rate was 56% but 85% in the group of age>50 yrs. OGTT 75 g, anthropometric measurements, cholesterol (total, HDL, LDL), triglicerides were measured. Adjusted prevalence-estimated error using fraction index were calculated.

Results: The overall prevalence of diabetes (DM) was 8.06±0.90% (male 7.15±1.25; female 8.94±1.30) including 5.56±0.77% of known DM and 2.52±0.56% of newly diagnosed DM. Respective figures for male were: known −5.56±1.13%, new −1.59±0.64%; and for female were: known −5.56±1.06% and new −3.37±0.89%. Impaired glucose tolerance (both IGT and IFG) was found additionally in 8.44±0.93% (male 6.09±1.17%; female 10.70±1.41%. After adding registered cases of diabetes in children and number of inhabitants at the age <18 yrs the prevalence of diabetes in overall population was calculated as 6.54±0.73% (4.59±0.62 known; 2.05±0.44% new) and impaired glucose tolerance as 6.85±0.68. The prevalence of metabolic syndrom (IDF criteria) was 34.1% in the group studied including 21.8% in normal glucose tolerant subjects; 76.5% in diabetics; 54.4% in IFG/IGT group.
Conclusion: Prevalence of diabetes in Southern Poland is higher than estimated indirectly but the percentage of undiagnosed diabetes is reduced. The prevalence of cardiovascular risk factors (metabolic syndrom) is similar to other caucasian populations.
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0310

Diabetes among immigrants from Turkey and Lebanon compared to Danes. Prevalence, treatment, screening for complications and cardiovascular outcome

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Background and Aims: Population survey data have shown globally increased prevalence rates of diabetes. For unknown reasons Europeans (Caucasians) may have a lower progression rate of Type 2 diabetes than patients from other parts of the world. By use of national and regional registries, this study aimed to identify trends in diabetes prevalence, treatment, care and cardiovascular complications among Lebanese (L) and Turkish (T) immigrants compared to native Danes (DK).
Materials and Methods: Patients with diabetes, their country of origin, laboratory measurements, medical treatment, frequency of examinations by ophthalmologists and cardiovascular complications were identified from The National Health Service Registry, The Regional Laboratory Database, The Danish National Hospital Registry and the Danish Civil Registration System. Patients were classified as Type 2 diabetics if they were treated with diet alone, with oral anti-diabetic agents or, irrespective of treatment, if they were over 40 years of age at the time of diagnosis (results from a questionnaire). Data were collected from 2000 through 2003 in a background population of 649,177 citizens in Aarhus County, corresponding to 12% of the total Danish population.
Results: Age-standardised prevalences of Type 2 diabetes were 18.9% (L, N=85); 19.8% (T, N=152) and 3.6% (DK, N=8885). In the period of 2000–2003 a considerable variation in the quality of glycaemic control both within and between ethnic groups was found. Thus, in 2003 HbA1c was measured at least once in 84% (DK), 87% (L) and 83% (T) of the patients. Furthermore, 72% (DK), 53% (L), and 51% (T) had an HbA1c <8% ($p<0.05$). In 2003, a large proportion of Danes were in non-pharmacological treatment: 40% (DK), 29% (T), and 32% (L) (DK vs. T, $p<0.05$), while immigrants were more likely to be treated with oral antidiabetic agents: 42% (DK), 53% (T), 53% (L); ($p<0.05$). There were no differences in the proportions of patients treated with insulin: 18% (DK), 19% (T), and 15% (T) (p=NS). During 2000–2003 increasing proportions of patients with total cholesterol <5.0 mmol/l were found in all groups: 37– 42% (L), 41–48% (T), and 34–47% (DK) (p=NS). This corresponds with an increase in the use of lipid-lowering treatment: 17–27% (L), 13–32% (T), and 18–38% (DK) (DK and L ; $p<0.05$). In 39% (DK), 35% (L), and 37% (T) of the patients no examination for retinopathy was registered during the period of 2000–2003. A cardiovascular diagnosis was registered in 27% (DK), 32% (L) and 33% (T) of the patients (p=NS).
Conclusion: Compared to native Danes, Lebanese and Turkish immigrants had a much higher prevalence of Type 2 diabetes and worse glycaemic regulation. The routine monitoring of diabetes care for native Danes, Lebanese or Turkish immigrants was comparable. Still, there is room for improvement in complication screening programmes. Despite differences in glycaemic control, cardiovascular complications were equivalent in the three groups.

0311

High prevalence of undiagnosed type 2 diabetes, other types of impaired glucose regulation and associated cardiovascular risk factors among working population of Chelyabinsk

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Background and Aims: It is known that great proportion of patients (pts) with type 2 diabetes (DM 2) remains

undiagnosed for several yrs resulting in high prevalence of cardiovascular (CV) complications at the time of diagnosis. The aim of the study was to estimate the prevalence of DM 2, impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and associated CV risk factors among working population of Chelyabinsk aged 40 yrs and older.

Materials and Methods: Chelyabinsk is a big industrial city with the total population of 1 095 053 and population aged 40 yrs and older of 493963 (197533 males/296430 females). We examined a total of 4238 (1661 m/2577 f) randomly selected workers of 15 enterprises and institutions of different types, located in all 7 administrative districts of the city, aged 40–70, mean age±SD 52.0±6.8 yrs. Fasting capillary glucose (FCG) was analysed with a glucometer Accu–Chek Active (Roche Diagnostics) and in people with FCG 5.6–6.0 standard oral glucose tolerance test was performed. Weight, height, waist circumference (WC), systolic (SBP) and diastolic blood pressure (DBP) were measured and body mass index (BMI) was calculated. Prevalence data are shown as mean%±95%CI. General linear univariate model with age-adjustment was used to compare prevalence rates.

Results: According to WHO (1999) criteria the prevalence of isolated IFG and combined IFG/IGT was 7.9 [7.1–8.7] and 1.8 [1.4–2.2]%, respectively. The estimated prevalence of previously and newly diagnosed DM2 was 2.1 [1.6–2.5] and 5.9 [5.2–6.6]%, respectively. The estimated number needed to screen (NNTS) to identify one person with previously unknown dysglycaemia was 6.4 [6.0–6.9].

Age-adjusted prevalence of obesity (BMI≥30 kg/m^2), abdominal obesity (WC>88 cm in women, WC>102 cm in men) and arterial hypertension (SBP≥140 and/or DBP≥90 mm Hg) was significantly higher in all groups with abnormal glucose level compared to normoglycaemic individuals ($p<0.001$) but no significant difference ($p>0.05$) was found when compared isolated IFG, combined IFG/IGT and newly diagnosed DM2 groups with previously diagnosed DM2 pts (table 1).

Conclusion: Screening among working population aged 40 yrs and older is an efficient method to identify dysglycaemic subjects. The prevalence of undiagnosed DM2 among working residents of Chelyabinsk is almost 3 times higher than the prevalence of registered DM 2. Any type of impaired glucose regulation was associated with higher prevalence of CV risk factors. Subjects with undiagnosed DM 2 and intermediate glucose abnormalities demonstrated CV risk profile similar to pts with previously diagnosed DM2.

Table 1. Age-adjusted prevalence of CV risk factors according to the categories of dysglycaemia in the study population.

Risk factor	Normal FCG	IFG	IFG +IGT	Previously diagnosed DM2	Undiagnosed DM2
n	3491	332	77	88	250
Obesity	27.8% [26.3–29.2]	40.2% [34.9–45.5]*	51.6% [40.2–63.0]*	54.5% [43.9–65.1]*	57.1% [51.0–63.3]*§
Abdominal obesity	25.9% [24.5–27.4]	45.5% [40.1–50.9]*	54.4% [43.0–65.8]*	56.9% [46.3–67.4]*	56.9% [50.7–63.0]*#
Arterial hypertension	28.5% [27.0–30.0]	41.9% [36.6–47.2]*	41.7% [30.4–52.9]*	44.9% [34.3–55.5]*	53.5% [47.3–59.7]*#

* p<0,001 compared to normal FCG
§ p<0.001 compared to IFG
# p<0.01 compared to IFG

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0312

Gender differences in the prevalence of impaired fasting glucose (IFG) in healthy Thai

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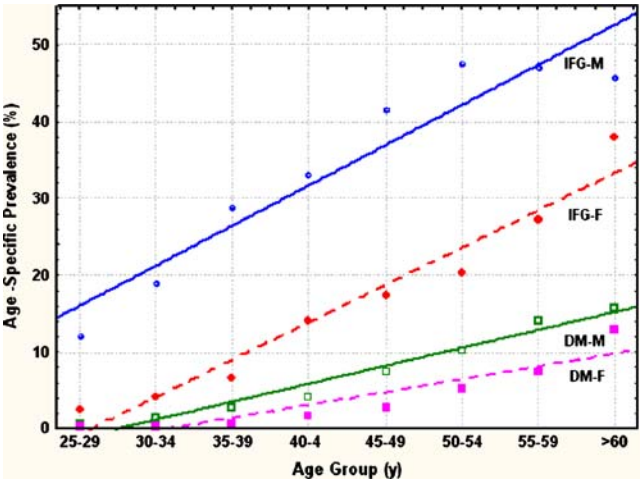
Background and Aims: A substantial number of patients are unaware of their glycemic status and have already developed chronic complications at the time of the diagnosis of hyperglycemia. These undiagnosed patients can only be discovered by fasting plasma glucose (FG) measurement. Individuals with IFG are predisposed for DM and metabolic syndrome. Screening these individuals is essential for early identification and management of DM. However, the prevalence of IFG by age and gender using the recent ADA classification in healthy Thai is not known.

Materials and Methods: We retrospectively reviewed FGs in Thai in one tertiary care medical center from 1/2002–12/2004. FG was determined by glucose oxidase method. Individuals were categorized to 8 age groups at 5-year interval, beginning from 25 to>60 years old (y). The age-specific FG was calculated to represent the average FG of each age group. The population estimated was based on Thailand 2002 census (41.4 million people aged ≥25 y). Data were presented as mean±SD.

Results: There were 14837 patients, most were middle class and resided in metropolitan area. None is known to

have prior history of hyperglycemia. Forty six percent were male. The mean age was 43±11 (25–84) y for male and was 42±11 (25–84) y for female. FG increased with age in both genders with the predicted incremental rates of 0.67 and 0.62 mg/dl/year for male and female respectively (r=0.30 for male and r=0.36 for female, p<0.001 each). As a group, FG was elevated in male compared with female (101±25 mg/dl vs. 93±19 mg/dl, P<0.001). In addition, the age-specific FG was also higher in male (ANCOVA, P<0.01). For Thai aged ≥ 25 y, the overall estimated prevalence was 22.2% for IFG (8.3 million people) and 4.4% for undiagnosed DM (1.8 million people). As with FG, the undiagnosed hyperglycemia (IFG and undiagnosed DM) were more common in older individuals in both genders (r=0.98, p<0.001, each, figure 1). When adjusted for age, the undiagnosed hyperglycemia was again more prevalent in male than female (ANCOVA, P<0.01).

Conclusion: Our study has observed the influence of age and gender on plasma glucose and the prevalence of hyperglycemia in Thai. The epidemic of the undiagnosed hyperglycemia is an important public health concern. This information is essential for establishing a screening program to control and prevent DM and its long term complications.



0313

Prevalence of diabetes, impaired fasting glucose and obesity in Iran

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Background and Aims: Obesity and diabetes currently threaten the health, well-being and economic welfare of virtually every country in the world. The purpose of this study was to estimate the prevalence of diabetes (DM), impaired fasting glucose (IFG) and obesity and the association of DM& IFG with risk factors in the Iranian population.

Materials and Methods: The Iranian National Health and Nutrition Surveillance system 2004– 2005 was a nationally representative survey with multistage cluster sampling design. Data from a comprehensive questionnaire, a physical examination, and blood tests were obtained from 52519 Iranian adults in urban and rural areas (25244 men and 27275 women) aged 25–65 years.

Results: The age-adjusted prevalence of diabetes in this population was 5.9%, and the age-adjusted prevalence of previously diagnosed diabetes was 2.7%. Overall, these results indicate that 5.6% of Iranian men and 6.1% of Iranian women have diabetes. The age-adjusted prevalence of IFG was 15.4%, using the new American Diabetes Association criteria. The overall prevalence of overweight (BMI 25–29.9), obesity (BMI>30) and morbid obesity (BMI>35) were 28.6%, 10.8% and 3.4% respectively. The prevalence of obesity was significantly higher in women than men (14% versus 7.4% P<0.001). Diabetes and IFG prevalence increased with age and peaked in the oldest age-group. Diabetes was found to be associated with age, BMI, waist circumference, smoking, blood pressure, cholesterol, exercise, education, high caloric diet and a family history of diabetes.

Conclusion: This study shows that diabetes, IFG and obesity are common in Iran, and about one-half of diabetes cases remain undiagnosed. These results emphasize the need to develop an urgent public program to improve the detection, prevention, and treatment of diabetes.

Supported by MOH

0314

Phenotypic characteristics among young adult-onset diabetic subjects in Sri Lanka (A South Asian population) – Sri Lanka Young Diabetes Study (SLYDS)

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Background and Aims: Diabetes mellitus is rapidly becoming a public health problem among South Asians. Clinical phenotypes among young adults are not well characterised. We aimed to characterise the different clinical

phenotypes among young adult onset diabetic subjects with a view to identifying phenotypes that need different clinical management and primary preventive strategies.

Materials and Methods: Cross sectional descriptive study. Ethical approval obtained from the Ethical Review Committee of the Faculty of Medicine, Univrsity of Colombo. Diabetic subjects who were diagnosed between 16 to 40 years of age were recruited from free government clinics and from fee levying private sector clinics. Data collection conducted at the Diabetes research Unit of the University of Colombo in Sri Lanka. Detailed clinical and metabolic assessments were undertaken using a prevalidated case record form by trained medical graduates according to standard methods. Lipid analysis done using Hitachi 704 analyser and HBA1c in BIO–RAD HPLC analyser. Statistical analysis was performed using SPSS version 13.

Results: Total numbers of subjects 1007, mean age 36.62 years (SD 5.82), mean age at diagnosis 31.87 years (SD 5.62), females 57.8%, and mean diabetes duration 4.81 years (SD 4.18). Overall, 16.4% of subjects are treated with insulin. The percentage that was treated with insulin from the onset or due to primary failure of oral therapy is 12.4%. The mean body mass index is 24.64 kg/m^2. According to the cut-off values for South Asians (23 kg/m^2), 64.8% are overweight or obese. Central obesity is significantly higher among females; males 43.5% and females 80.4% ($p<0.001$). Acanthosis Nigricans is present in 20.3% of subjects. In this sample 73% has positive first degree family history and 9.6% has family history among three consecutive generations. Among females who had been pregnant, 22.32% gives a history of gestational diabetes mellitus. Incidence of diagnosed Polycystic Ovarian Disease is 0.87%. The mean HBA1c is 8% (SD 2). HDL Cholesterol is lower in 66.9% and Triglycerides high among 36.7% of subjects. The LDL is elevated in 74.8% and 94.2% have some lipid abnormality.

Conclusion: In this sample Type 2 diabetes is the predominant clinical phenotype. The prevalence of overweight and obesity is high. Females are more centrally obese than males and a high incidence of gestational diabetes is reported among the parous females. Most subjects had abnormal lipid profiles. Insulin resistance, islet cell antibodies and markers for monogenic diabetes are being analysed to refine further the aetiological diagnosis. Prospective follow up is being undertaken to define the natural history among different phenotypes.

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0315

Population-based incidence of type 2 diabetes in Asturias, Spain

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Background and Aims: Type 2 diabetes (DM) is reaching epidemic proportions worldwide and for prevention purposes, identification of persons at high risk is mandatory. The aim of the present study was to define the incidence of type 2 diabetes in a Caucasian low-risk population in the north of Spain and its relationship with impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and other risk factors.

Materials and Methods: The "Asturias Study" is a prospective population-based survey of diabetes and cardiovascular risk factors. The baseline examination was carried out during 1998–1999 when 1034 individuals aged 30–75 years were randomly selected to determine the prevalence of DM and prediabetes in the region of Asturias (north of Spain). The results showed that 11.3% of all participants had diabetes, 12.4% ITG and 7.6% IFG.

In 2004–2005, these same subjects were invited for a follow-up examination. 42 of them had died and 19 had moved out of Asturias. Of the remaining 973, 700 participated (72%). For the present study we include only the 630 individuals without diabetes at baseline.Glucose was measured in venous blood both fasting (FPG) and 2 h after 75 gr oral glucose tolerance test (2hPG). We used WHO 1999 criteria to classify glucose metabolism at both baseline and follow-up: IFG:FPG 110–125 mg/dl, IGT: 2hPG 140–199 mg/dl, DM: FPG ≥126 mg/dl and/or 2hPG ≥200 mg/dl.

Results: The study population includes 630 individuals aged 30–75 years at baseline and with an average follow-up of 6.3 years. During follow-up, 44 of them developed diabetes (7%). The incidence of diabetes adjusted to age and sex structure of Asturias is 10.8 cases per 1000 persons/years. Cumulative incidence of diabetes in prediabetic states are 36.5% for IFG, 23,9% for IGT and 60% for individuals with combined IFG–IGT. In a multivariate logistic regression analysis, in addition to IFG and IGT, hypertrigliceridemia (p 0.000) and diastolic hypertension (0.002) were also independently associated with development of DM.

Conclusions: Our study shows in this population of the north of Spain an incidence rate of type 2 diabetes of 10.8 cases per 1000 persons/years. IFG as defined by the WHO 1999 was the strongest predictor of diabetes. The risk was extremely high for people with both IFG and IGT.
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0316

Exocrine and endocrine pancreatic function in type 2 diabetes patients of Bangladesh
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Background and Aims: Exocrine pancreatic dysfunction in type 2 diabetes is still an unsettled issue. Since *SPINK1* gene N34S variant has been associated with pancreatopathy we aimed to analyze the gene variant and evaluate the exocrine and endocrine functional status in a cohort of typical type 2 diabetic patients in comparison to tropical calcific pancreatitis patients with and without diabetes.
Materials and Methods: Age-matched type 2 diabetic patients (n=49), tropical calcific pancreatitis without diabetes (TCP, n=35) and with diabetes [Fibrocalculus calculus pancreatic diabetes (FCPD), n=83)] were studied. Exocrine function was evaluated by measurement of fecal elastase1 (by ELISA) and endocrine function by measurement of insulin (by RIA) following IV agrinine stimulation test. *SPINK1* gene N34S variant was analyzed by direct DNA sequencing. Student's unpaired-'t', Mann-Whitney and Chi-square tests were performed where applicable.
Results: Mean (±SD) BMI of the T2D (23.8±4.7) subjects was significantly higher compared to TCP (19.5±3.1, $p<0.001$) and FCPD (18.6±3.1, $p<0.001$) subjects. Fecal elastase1 was severely (<100 μg/g of stool) reduced in 58% of T2D subjects compared to 81% ($p=0.082$) of TCP and 95% ($p=0.001$) of FCPD subjects. *SPINK1* gene N34S variant was positive in 8.8% of T2D, 37% TCP ($p=0.011$) and 33% FCPD ($p=0.011$) subjects. *SPINK1* gene N34S variant positivity was present in subjects with severely reduced Fecal elastase1 of 15% of T2D, 18% TCP and 39% FCPD subjects. In all three groups *SPINK1* N34S variant positive and negative cases did not show any difference regarding Fecal elastase1. Insulin levels (median, U/l) at basal and on arginine stimulation state (at 15, 30, 45 and 60 min) in T2D (basal- 24.4, stimulated- 41.7, 42.3, 34.3 and 21.9) subjects was significantly higher compared to TCP (basal- 13.0, stimulated- 21.6, 23.4, 18.7 and 14.0) and FCPD (basal- 15.9, stimulated- 20.7, 19.5, 22.5 and 15.1) subjects. Between *SPINK1* gene variant positive and negative cases insulin levels at basal and stimulated state did not show any statistical difference.
Conclusion: The data suggest that i) a substantial proportion of type 2 diabetic patients in Bangladesh have exocrine pancreatic dysfunction; ii) in T2D the exocrine dysfunction and insulin secretory capacity is not associated with *SPINK1* gene variant.
The study was supported by grant from IPICS and DAB

PS 7

Prediction and prevention of type 2 diabetes and its complications

0317

Do fasting serum insulin and proinsulin levels in pre-diabetic individuals identified in a high-risk screening programme predict progression to diabetes. The ADDITION study (DK)
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Background and Aims: Persons identified with pre-diabetes (IFG and IGT) in a high-risk screening programme are at a particularly high risk of developing diabetes. Fasting serum proinsulin is a determinant for progression in population-based studies. We wanted to compare the level of fasting serum insulin in pre-diabetic individuals identified in a high-risk screening programme with that in individuals identified by mass-screening. Secondly, we wanted to examine whether fasting serum insulin and proinsulin or proinsulin/insulin ratio are predictors for progression to diabetes after one year in high-risk screened pre-diabetic individuals.

Materials and Methods: A high-risk screening programme, the ADDITION study Denmark, identified 699 individuals with IFG and IGT (WHO1999 definition), in whom there were one-year follow-up data. The Inter99 study, a mass-screening study in Denmark, identified 1,637 individuals with IFG and IGT. Baseline fasting serum insulin levels in these populations were compared. Poisson regression was used in the ADDITION population to estimate rate ratios of progression to diabetes between levels of fasting serum insulin, serum proinsulin and serum proinsulin/insulin ratio. Secondly, the model was adjusted for screening variables (age, sex, family history of diabetes, gestational diabetes, known hypertension, BMI, physical activity, random blood glucose, HbA1c), glucose tolerance status, and systolic blood pressure. Serum insulin was determined by ELISA with a narrow specificity excluding Des(31,32)- and intact proinsulin and serum proinsulin was determined by ELISA with a broad specificity including 4 proinsulin conversion intermediates (65–99%) together with intact proinsulin (100%).
Results: Fasting serum insulin levels in the ADDITION and the Inter99 populations were (median) 50.0 and 45.0 pmol/l, respectively ($p<0.0001$). In the ADDITION study, the rate ratios of progression from pre-diabetes to diabetes associated with a two-fold increase were: insulin 1.13 ($p=0.09$), proinsulin 1.30 ($p<0.001$) and proinsulin/insulin ratio 1.52 ($p<0.001$). After adjustment, these associations diminished and became insignificant: insulin 1.00 [95% CI:0.84–1.18] ($p=0.98$), proinsulin 1.09 [0.92–1.30] ($p=0.34$), proinsulin/insulin ratio 1.26 [0.95–1.67] ($p=0.10$). Glucose tolerance status was not significantly associated with progression.
Conclusion: Pre-diabetic individuals in high-risk screening programmes are more insulin resistant as estimated from fasting serum insulin levels than those in classical surveys. In our high-risk screened population, fasting serum insulin and proinsulin or proinsulin/insulin ratio did not predict progression to diabetes when adjusted for study design, glucose tolerance status and systolic blood pressure.
This fully investigator initiated, designed and controlled study received funding from a number of public as well as private sources.

0318

Carbohydrate and fiber intake and incidence of type 2 diabetes in the EPIC-Potsdam Study
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Background and Aims: Although some prospective studies have evaluated whether intake of carbohydrates is associated with risk for type 2 diabetes, it remains unclear what long-term effects substituting protein at the expense of carbohydrates, common with popular low-carbohydrate protein-rich diets, may have with regard to diabetes risk. Our objective was to evaluate carbohydrate intake in predicting type 2 diabetes using substitution models for fat and protein and to evaluate whether fiber intake is associated with risk.
Materials and Methods: We conducted a prospective cohort study of 9,711 men and 15,402 women aged 35–65 years and free of diabetes who were followed for incident type 2 diabetes. Dietary intake of macronutrients and fiber were estimated with a validated food-frequency questionnaire. We estimated the relative risk (RR) using Cox proportional hazards analysis. Because there were no significant interactions with sex, we pooled men and women in all analyses.
Results: During 176,780 person–years of follow-up we observed 849 incident cases of physician confirmed type 2 diabetes. There was no significant association between carbohydrate intake and diabetes risk in models substituting for total fat. After adjustment for age, BMI, waist circumference, potential lifestyle confounders, soluble fiber, insoluble fiber, alcohol, and magnesium intake, the relative risk for substituting 5% of energy intake from total fat with carbohydrates was 1.03 (95% KI: 0.95–1.11). In contrast, substituting carbohydrates for protein significantly reduced diabetes risk (RR for a 5% energy substitution: 0.74; 95% CI: 0.63–0.88). This association was similar across strata of carbohydrate intake (<vs.>=45% energy) and obesity (BMI <vs.>=30). A non-significant inverse association with diabetes risk was observed for total fiber intake (RR for extreme quintiles of energy adjusted intake: 0.81 [95% CI: 0.62–1.06]). However, higher cereal fiber intake was significantly associated with a reduced diabetes risk independent of other fiber sources (RR for extreme quintiles of energy adjusted intake: 0.70 [95% CI: 0.55–0.89]), while fruit fiber (0.87 [0.68–1.10]) and vegetable fiber (0.95 [0.76–1.20]) were not significantly associated. Among carbohydrate-rich foods, whole-grain bread intake was associated with reduced risk of developing diabetes. The RR for the highest quintile compared to the lowest was 0.79 (95% CI: 0.64–0.99).
Conclusion: Substituting carbohydrates for fat appears not to be related to diabetes risk. However, a higher protein intake at the expense of carbohydrates may increase diabetes risk suggesting potential harmful effects of popular low-carbohydrate protein-rich diets. Cereal fiber and whole-grain bread intake may reduce diabetes risk.
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0319

Is measurement of HbA_{1c} alone appropriate as an initial screening test for undiagnosed diabetes?

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Background and Aims: Glycated hemoglobin A1c (HbA1c) is currently used to estimate number of people with diabetes (DM) in the Japan National Diabetes Survey (JNDS). In the JNDS, the possible DM was defined as people with HbA1c ≥6.1% or under treatment of DM and the probable DM was defined as those with HbA1c 5.6–6.0% in people without treatment of DM. According to the JNDS in 2002, the total number of people having probable and possible DM was estimated as 7.4 million and 8.8 million in the whole population over 20 years old. The aim of this study is to assess the efficacy of HbA1c as an initial screening test for undiagnosed DM, by using population-based data from the Funagata study in 2000–2002.
Subjects and Methods: 1,904 Funagata-town inhabitants aged 35–89 years old were analysed. HbA1c was measured together with plasma glucose during an oral glucose tolerance test and DM was diagnosed according to the 1999-WHO criteria. The area under the receiver operating characteristic (ROC) curve predicting undiagnosed DM was compared between HbA1c and fasting plasma glucose. Screening properties for HbA1c predicting undiagnosed DM was examined, with respect to sensitivity, specificity, predictive values positive (PPV) and negative (NPV), and the proportion of people above the cut-off point. Moreover, impact of sex, age, and BMI on the ROC curve of HbA1c for undiagnosed DM was examined.
Results: In the Funagata study, the prevalence of previous DM, undiagnosed DM, and Impaired Glucose Regulation according to the WHO criteria were: 5.5%, 5.8%, and 18.8% in the population. The area under the ROC curve for undiagnosed DM was similar between HbA1c (0.849 [95% Cl: 0.814–0.895]) and fasting plasma glucose (0.899 [0.864–0.933]).The area under the ROC curve of HbA1c for undiagnosed DM had a trend to increase with increase of BMI and decrease of age, with statistical insignificance. HbA1c of 5.6% gave a sensitivity of 54.9%, a specificity of 94.9%, a PPV of 41.5%, a NPV of 97.0%, and the proportion of people above the cut-off point of 8.2%. At HbA1c of 5.3% where maximizing sensitivity plus specificity, the corresponding values were: 76.6%, 81.9%, 21.7%, 98.2%, and 21.7%. The PPV, 1-NPV, and the proportion of people above the cut-off level increased with increase of HbA1c. The current cut-off point of 5.6% was lower than the points where maximizing sensitivity plus specificity predicting retinopathy in the future among Japanese general population (HbA1c: 5.7%) and the treatment target of HbA1c recommended by the guideline in Japan (HbA1c: 5.8%).
Conclusions: HbA1c seems to be a useful initial screening test for undiagnosed DM and the current cut-off point of 5.6% may be appropriate, with respect to screening properties for undiagnosed DM, and prediction and prevention of microvascular complications in Japan.
The analysis of this study was supported by the grant from the Japanese Ministry of Health, Welfare and Labour.

0320

Childhood predictors of young onset type 2 diabetes mellitus

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Background: Optimal strategies for preventing young onset type 2 diabetes mellitus (T2D) require the identification of the disease's early-life modifiable risk factors. We aimed to do this using longitudinal data in 1,604 American Indian children and adolescents who were nondiabetic at baseline (5–19 yrs; 5.7% developed T2D during follow-up).
Methods: For T2D prediction, we derived an optimally-weighted continuously distributed standardized multivariate score (zMS) comprised of commonly measured metabolic risk factors (i.e., fasting and 2 hr glucose, HbA_{1c}, BMI, waist circumference, fasting insulin, HDL-C, triglycerides, and systolic and diastolic blood pressures), and compared the predictive power for each feature against zMS using ROC analyses.
Results: In separate Cox proportional hazard models, adjusted for age, sex, and fraction Indian heritage, associations with incident T2D were observed for all metabolic risk factors. Stepwise proportional hazards regression models selected fasting glucose, 2 hr glucose, HDL-C, and waist as independent predictors of T2D, but individually these were weaker predictors than zMS ($p<0.01$). However, a parsimonious summary score constructed from only these variables had similar predictive power as zMS (ROC AUC: 0.77 vs. 0.78; $p=0.10$). Although intrauterine exposure to diabetes or parental history of early onset T2D substantially increased a

child's absolute risk of developing T2D, the magnitude of the T2D risk relationships for zMS and the parsimonious score were no stronger than in children without these familial risk factors.

Conclusions: It may be possible to accurately predict young onset T2D using simple and non-invasive measures of fasting glucose, 2 hr glucose, HDL-C, and waist. The predictors of T2D differ in younger and older children possibly because of changes in metabolism that occur during puberty. These data may prove informative for strategies that seek to target the early-life risk factors for T2D. They also point to the features of a childhood-specific metabolic syndrome score that are relevant for T2D prediction.

Table. Pair-wise comparisons of the full model (zMS) with each parsimonious model derived from a step-wise regression procedure.

	Model	AUC	(SE)	p-value
5–9 yr olds	1. Full model (zMS)	0.90	(0.02)	–
	2. Waist	0.88	(0.03)	0.17
10–14 yr old	1. Full model (zMS)	0.82	(0.03)	–
	2. BMI, HbA_{1c}, 2 hr gluc	0.79	(0.03)	0.06
15–19 yr olds	1. Full model (zMS)	0.76	(0.03)	–
	2. Waist, HbA_{1c}, 2 hr gluc	0.74	(0.03)	0.04
5–19 yr olds	1. Full model (zMS)	0.78	(0.02)	–
	2. Fast gluc, 2 hr gluc, BMI, HDL-C	0.77	(0.02)	0.10

P-value is for pair-wise comparison of Full model with Partial model. All models include terms for age, sex and fraction Indian heritage. BMI=body mass index; Fast gluc=fasting glucose; 2 hr gluc=2 hr hour glucose; HDL=high density lipoprotein cholesterol. Predictor variables were standardised (z-score) within one year age strata.

Parsimoniously predictive subsets of the variables (shown above as model 2) were identified by a backward stepwise procedure. All predictor variables were included in the initial model and the weakest predictor was sequentially eliminated until all of the remaining variables were retained at $p<0.05$; (age, sex and heritage were not made available to the stepwise procedure and are, thus, forcibly included in all models). To assess the extent that this submodel captured the information contained in the fully optimized model containing all variables, the ROC AUCs for these two models were compared (difference between models indicated by p-values in table).

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0321

Smoking and the risk of type 2 diabetes mellitus

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Background and Aims: The aim of study was to determine the association between smoking and the risk of type 2 diabetes mellitus.

Materials and Methods: A case-control study included 234 cases with newly confirmed diagnose of type 2 diabetes mellitus and 468 controls who were free of the disease in 2001. Cases and controls were matched by gender and age (+5 yr.). Information on age, gender, body mass index, family history on diabetes, education level, years of total education, occupation status, nutrition habits, alcohol consumption, cigarette smoking, physical activity and stress was collected from questionnaire. Conditional logistic regression was used to compute the odds ratio (OR), 95% confidence intervals (CI) and p for trend. The ORs were adjusted for potentials confounder such as: family history on diabetes, body mass index, speed of eating, portion of food, using of salty and fatty food, physical activity early in life, exercise, years of total education, and stress that were related to type 2 diabetes mellitus in univariate regression model and alcohol drinking, that were associated with the disease.

Results: After adjustment for possible confounders increased risk of type 2 diabetes mellitus determined for current smokers (OR=2.25; 95% CI 1.03–4.92), duration of smoking (OR=2.43; 95% CI 1.14–5.20) for 20–39 yrs, compared with never-smokers. There were no statistically significant associations between risks of type 2 diabetes mellitus, and number of cigarettes smoked a day, number of pack-years and time after quit up smoking.

Conclusion: Our data suggest that smoking is a significant risk factor of type 2 diabetes mellitus.

0322

Microalbuminuria predicts diabetes mellitus in men from the general population

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Background: Microalbuminuria (Malb) is associated to metabolic syndrome, hypertension and many other cardio-

vascular risks. Whether elevated Malb constitutes a risk for diabetes mellitus is presently unknown.
Objectives: We prospectively assessed the predictive value of Malb for the development of diabetes mellitus in men and women selected from the general population.
Subjects and Methods: 3252 non diabetic subjects (aged 30 to 64 years) were included in the D.E.S.I.R. Study. At baseline, the subjects had a complete medical and biological checkup. After a 9-year follow-up, new cases of diabetes mellitus were recorded.
Results: The 9-year incidence of diabetes mellitus was 4.6%; it occurred more frequently in men (6.8%) than in women (2.0%). Diabetes mellitus was more frequent in microalbuminuric than in normoalbuminuric subjects (RR: 2.00 [1.29–3.10], P=0.0020). The risk associated to microalbuminuria was greater in men (2.11 [1.29–3.44], P=0.0030); however, it did not reach the significance threshold in women (1.44 [0.50–4.19], P=0.4986). The risk of diabetes mellitus associated with microalbuminuria in men was unchanged despite multiple adjustments.
Conclusions: Microalbuminuria is a strong, independent risk for diabetes mellitus in men.
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0323

Clinical and cost-effectiveness of primary prevention of type 2 diabetes in a routine health care setting: Model based on the KORA Survey 2000

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Background and Aims: Lifestyle modification and Metformin have been demonstrated to prevent progression from prediabetic status to type 2 diabetes (Diabetes Prevention Program (DPP)). Several studies have analyzed costs and cost effectiveness of type 2 diabetes prevention, and found both medication and lifestyle modification to be cost effective. However, so far, no analysis considered an implementation in a routine health care setting. In particular, a high participation of patients in the prevention interventions was assumed, which might be unrealistic in clinical practice. The aim of our study was to evaluate the clinical and cost-effectiveness of metformin and lifestyle intervention to prevent type 2 diabetes in an elderly high risk population, which was identified by a targeted screening, in a routine health care setting, using population-based data (KORA-Survey in Augsburg, Germany, whole population about 600,000).
Material and Methods: We performed a decision analytic model with a time horizon three years. Interventions were staff education, targeted screening, and lifestyle modification or metformin in subjects aged 60–74 years with BMI>=24 and prediabetic status (target population, about 72,500), according to the Diabetes Prevention Program trial. Main outcome measures were cases of type 2 diabetes prevented or delayed, costs (Euro), incremental costs per additionally avoided case of diabetes (incremental cost-effectiveness ratios, ICER's), all from third party payers' and societal perspectives.
Results: Under model assumptions, 14,908 subjects of the target population would develop diabetes without intervention. 184 and 42 cases would be avoided with lifestyle and metformin intervention, respectively. Costs were Euro 856,507 and Euro 4,961,340 for lifestyle modification and Euro 797,539 and Euro 1,335,204 for metformin from statutory health insurance' and societal perspective. Up to 5% of costs were due to staff education, and up to 36% to screening. Lifestyle was more cost effective than metformin. ICERs for lifestyle versus no intervention were Euro 4,664 and Euro 27,015 per case prevented from the statutory health insurance' and societal perspective.
Conclusions: Total costs and costs per diabetic case avoided were high, and staff education and screening had a considerable impact. Considering low participation in a routine health care setting, with both strategies, there was only a small number of diabetic cases that would be prevented or delayed. Before implementing a program, more effort should be undertaken to improve patient participation to achieve more clinical and cost effectiveness in type 2 diabetes prevention in clinical practice.

0324

Feasibility and uptake of screening for type 2 diabetes in primary care

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Background and Aims: Uncertainty remains over whether screening for type 2 diabetes is cost-effective. Screening can only be cost-effective if there is sufficient uptake. Identification of those with poor uptake can facilitate appropriate targeting of screening programmes. The

ADDITION Study (Cambridge) is a randomised trial of a programme of screening and intensive multifactorial intervention for type 2 diabetes in primary care in the east of England. This investigation aimed to determine the factors influencing screening uptake.

Materials and Methods: A validated risk score based on age, sex, body mass index (BMI) and prescribed steroid and antihypertensive medication was calculated from information held in general practice notes for people aged 40–69 years without known diabetes. Those with a risk score above a cut-off equivalent to the top 25% of the population distribution were invited to attend an appointment at their practice for a random capillary blood glucose (RBG) test (HemoCue) and HbA_{1c} test (Biorad). Participants with an RBG of ≥11.1 mmol/l were invited for a standard 75 g oral glucose tolerance test (OGTT). Those with a RBG of 5.5–11.1 mmol/l were invited to return for a fasting capillary blood glucose (FBG) test. Those with a FBG of ≥6.1 mmol/l, or a FBG of 5.5–6.1 mmol/l and an HbA_{1c} of ≥6.1%, were invited for an OGTT. By the end of June 2005, 33 general practices had completed the screening process and were included in these analyses. There were 76,574 patients between the ages of 40 and 69 years in these practices representing 33.5% of the overall practice population. The prevalence of known diabetes in this age category was 3.9% with a range of 2.1 to 6.1%. The proportion of 40–69 year olds without a calculated risk score was 27.2% (range – 10.3 to 67.3%). The mean (SD) BMI was 26.5 (5.0) kg/m^2.

Results: Of the 17 814 with a high risk score and invited for an RBG test 77% attended. Screening uptake increased to 93% for the subsequent two stages (FBG testing and diagnostic OGTT). A total of 4930 were eligible for FBG and 846 for OGTT. There were 503 screen-detected cases of diabetes (3.7% of those attending the RBG test). Screening uptake for the RBG test was significantly higher among older people, women and those with a high BMI and a high risk score (p-value <0.001 for all comparisons) while age ($p<0.001$) and RBG result ($p=0.009$) were associated with uptake of the FBG test. The practice level factors investigated (practice size, prevalence of known diabetes, urban–rural location, and proportion with missing data on risk score variables) were not consistently associated with screening uptake.

Conclusion: Targeted RBG screening of individuals assessed as high risk by data routinely available in general practice is feasible and acceptable. Uptake of screening was highest amongst those individuals at highest risk by virtue of age and BMI, but could not be predicted by practice level characteristics. Different strategies may be required to increase uptake of screening among younger men, should screening be shown to be beneficial.

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0325

Basic characteristics and treatment regimens in people with type 2 diabetes in the international diabetes management practices study (IDMPS)

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Background and Aims: The IDMPS is a multinational observational study that aims to gather data that reflect current care practices of people with diabetes in different areas of the world and to evaluate the compliance with international care guidelines.

Materials and Methods: Data are being collected from type 2 diabetic patients (≥18 years) in 1-year cycles (a 2-week cross-sectional period followed by a 9-month longitudinal period) for 5 years. Data from the first cross-sectional period are currently available from 7543 people (48% men) in 11 countries in Eastern Europe, Asia, and Latin America.

Results: The mean age was 58±11 years, mean BMI 27±5 kg/m^2 (men: 26±4 kg/m^2, women: 27±5 kg/m^2) and mean disease duration 8 years. Mean HbA_{1c} was 7.7±1.75% and 38% of people had an HbA_{1c} <7%. Among people with HbA_{1c} <7%, 4% were treated with diet and exercise only, 77% with oral antihyperglyemic agents (OHAs) alone and 19% with insulin±OHAs. A regimen of diet and exercise was reported in 3% of people (mean HbA_{1c} of 6.9±1.67%). OHAs alone were used by 67% of people (mean HbA_{1c} of 7.4±1.60%). Insulin±OHAs were used by 16% of people (average insulin dose 36 IU, mean HbA_{1c} of 8.3±1.89%). Mean FBG (laboratory) was 152±60 mg/dL. Mean systolic/diastolic blood pressure was 135/81 mmHg. Target treatment values were obtained for LDL (<100 mg/dL) in 34% of people, HDL (>40 mg/dL) in 64% of people, and triglycerides (<150 mg/dL) in 48% of people.

Conclusion: As can be seen, despite the evidence provided by the UKPDS and the recommendations of the ADA, the majority of people did not achieve the recommended

treatment goals for HbA_{1c} and FBG. It is thus apparent that more efficient guidance and education be made available to improve treatment outcomes in people with type 2 diabetes world wide.
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0326

WITHDRAWN

PS 8

Type 2 diabetes genes

0327

The combined impact of replicated hyperglycaemia susceptibility alleles on fasting and stimulated glycaemia in the general population: a study of 5,603 middle-aged treatment-naïve subjects

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Background and Aims: Previous population studies have below the threshold for diabetes shown a graded relationship between fasting and postprandial plasma glucose concentrations and the subsequent incidence of cardiovascular morbidity and mortality. Insights into the genetic determinants of this relationship may therefore be of relevance in identifying subgroups of the general population at high risk and in targeting genotype-based intervention against glycaemia induced morbidity. Four polymorphisms in *GCK* (−30 G>A, rs1799884), *HNF4A* (rs1884614), *KCNJ11* (Glu23Lys, rs5219), and *TFAP2B* (IVS1+774G>T), respectively, that are reported to associate with hyperglycaemia were selected for this study. The objective was at the population level to elucidate the combined effect of these risk alleles on fasting and post-OGTT plasma glucose levels.
Materials and Methods: The four polymorphisms were genotyped in the population-based Inter99 cohort of 5,603 middle-aged individuals (4,238 subjects with normal glucose tolerance, 478 subjects with impaired fasting glycaemia, 649 subjects with impaired glucose tolerance, and 238 screen-detected and treatment-naïve type 2 diabetic patients). The same polymorphisms were genotyped in a case-control study of 1,023 type 2 diabetic patients and 4,338 glucose-tolerant subjects. For each of the four polymorphisms the allele that was reported to contribute to hyperglycaemia was defined as the risk allele. We assumed that each risk allele has an additive effect and that the effect of all risk alleles is the same. A general linear model and a binary logistic regression analysis were used to estimate the effect of the risk alleles on glycaemic levels and prevalence of type 2 diabetes, respectively.
Results: Among 5,603 middle-aged treatment-naïve Inter99 participants a relationship between the additive effect of risk alleles and increased fasting plasma glucose levels was observed with an effect size of 0.059 (mmol/l) per risk allele ($p<0.0001$). This same relationship was observed for 30 min. and 120 min. post-OGTT plasma glucose levels with effect sizes of 0.117 (mmol/l) ($p<0.0001$) and 0.135 (mmol/l) ($p<0.0001$), respectively. Interestingly, in a study of 1,023 type 2 diabetic patients and 4,338 glucose-tolerant subjects the risk of having type 2 diabetes increased significantly as a function of the number of risk alleles with an odds ratio of 1.15 [95% CI 1.08–1.22] per risk allele ($p<0.0001$).
Conclusion: Combining susceptibility alleles which individually have been reported to associate with hyperglycaemia may be one important way to identify subjects in the general population at increased risk of developing premature cardiovascular morbidity and mortality.

0328

DUSP12 polymorphisms interact with the GCK −30G>A variant to increase fasting glycaemia in a large-scale population-based sample of middle-aged whites

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Background and Aims: DUSP12 is a dual-specificity phosphatase identified using a two-hybrid system assay as a protein capable of interacting with glucokinase. DUSP12 dephosphorylates glucokinase *in vitro* and in a dose-dependent manner accelerates its activity and hence may play an important role in glucose homeostasis. *DUSP12* is located on chromosome 1q21–q22, a region showing linkage to type 2 diabetes. We hypothesised that variation

in *DUSP12* associates with hyperglycaemia – either alone or in combination with a common −30G>A polymorphism in the gene encoding glucokinase (GCK).

Materials and Methods: The *DUSP12* rs1503814, rs1027702, rs1063178, and rs3820449 polymorphisms were genotyped in a population-based sample (Inter99) of 5,862 Danish whites (2,902 men, 2,960 women, age 46±8 years BMI 26.3±4.6 kg/m^2 (mean±SD)). All subjects underwent a standard 75 g OGTT.

Results: Applying a general linear model no association with fasting or post-OGTT plasma glucose levels was observed when analysing the *DUSP12* rs1503814 (minor allele frequency (MAF)=0.261), rs1027702 (MAF=0.371), rs1063178 (MAF=0.330), and rs3820449 (MAF=0.257) polymorphisms. Some degree of linkage disequilibrium was observed between the four *DUSP12* variants (pair-wise r^2 in the range of 0.4–0.8) and the most common haplotype had a frequency of 0.62; however, no association was observed with plasma glucose levels. Interestingly, an interaction was observed between the *DUSP12* variants and the *GCK* −30G>A polymorphism on fasting plasma glucose: p=0.04 (rs1027702) TT/AA 6.3±0.6 mmol/l, p=0.02 (rs3820449) TT/AA 6.3±0.8 mmol/l, and p=0.02 (rs1063178) TT/AA 6.4±0.6 mmol/l, whereas the remaining genotype combinations had fasting plasma glucose levels of 5.5±0.02 mmol/l.

Conclusion: *DUSP12* variation does not *per se* play a major role in the pathogenesis of hyperglycaemia in the general population of middle-aged whites. However, variants in *DUSP12* may in combination with the *GCK* −30G>A polymorphism contribute to the inter-individual variation in the fasting plasma glucose level.

0329

Polymorphisms of the Reg1α gene are associated with early onset type 2 diabetes in the Korean population

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Background and Aims: The *Reg* gene has been reported to be expressed in regenerating islets and Reg1 protein to be up-regulated early in the progression of type 2 diabetes in mice. As human *Reg*1α is homologous with murine Reg1, we investigated whether common variants in the *Reg*1α gene are associated with type 2 diabetes or with its related phenotypes in the Korean population.

Materials and Methods: We sequenced the *Reg*1α gene to identify common polymorphisms using 24 Korean DNA samples. Of 11 polymorphisms of the *Reg*1α gene found, five common ones (g.-385T>C, g.-36T>G, g.209G>T, g.1385C>G, and g.2199G>A) were genotyped in 770 type 2 diabetic patients and 643 nondiabetic subjects. In addition, the influence of the polymorphism on the promoter activity was investigated by electrophoretic mobility shift assays (EMSA).

Results: No polymorphism was found to be associated with the risk of type 2 diabetes. However, g.-385C and g.2199A lowered the risk of early-onset type 2 diabetes, which was defined as the first quartile in terms of the age at diagnosis (OR=0.750 [0.594–0.946] with P=0.015 and 0.737 [0.585–0.929] with P=0.023, respectively) and the haplotype consisting of g.-385C and g.2199A decreased the risk of early-onset diabetes (sex-adjusted OR=0.648 [0.469–0.894], P=0.008 in the dominant model). In addition, EMSA showed that NeuroD and upstream stimulating factor 1 (USF 1) specifically bound to the promoter region g.-385C of human *Reg*1α gene.

Conclusion:
Polymorphisms in the *Reg*1α gene were not found to be associated with overall susceptibility to type 2 diabetes, but were associated with early-onset type 2 diabetes in the Korean population possibly by modifying transcription activity.

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0330

The HNF-4A P2 promotor and risk of future type 2 diabetes

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Background and Aims: Mutations in the transcription factor Hepatocyte Nuclear Factor-4 alpha (*HNF-4α, TCF14)* gene is known to cause monogenic diabetes, Maturity Onset Diabetes of the Young type 1 (MODY1). *HNF-4*α is located on chromosome 20q12-q13, a region that has shown suggestive linkage to type 2 diabetes (T2D), and an important regulator in a network of transcription factors in the liver and pancreas. Variation in the *HNF-4*α, β-cell specific, P2 promoter have recently shown association to T2D. We hypothesized that variation in the P2-promoter will increase future risk of T2D.

Materials and Methods: Individuals in the Botnia Prospective (n=2293; 132 T2D converters during mean 6-year follow-up) and in the Malmö Preventive Project (MPP; n=4873; 491 T2D converters during mean 20-year follow-

up) cohorts were genotyped for three single nucleotide polymorphisms (SNPs) (rs4810424 [G/C], rs1884614 [C/T] and rs2144908 [G/A]) using the Taqman Allelic Discrimination technique on an Applied Biosystems 7900HT. Cohorts were analysed using multiple regression analysis at basal and last visits. The Cox proportional hazard model, applying a recessive genetic model, was used to predict future T2D. Analyses were adjusted for BMI, gender, age at visit and within family correlation when necessary.

Results: Genotype frequencies were in the same range as previously reported with minor allele frequencies at 18% and in Hardy-Weinberg equilibrium. The three SNPs were in linkage disequilibrium (D́>0.87, r2>0.75). Two of the three SNPs in the Botnia prospective cohort were associated with increased risk of T2D; rs4810424, HR=1.9 (95% CI 1.0–3.5; p=0.04) and rs1884614, HR=1.9 (1.0–3.4, p=0.04). In the MPP cohort the rs1884614 and the rs2144908 SNPs showed increased risk of future T2D; HR=1.8 (1.2–2.7, p=0.005) and HR=1.8 (1.2–2.7, p=0.004).

These differences were not associated with significant phenotypic differences (fasting plasma glucose, 2 h glucose, fasting insulin, 2 h insulin, HOMA–IR, HOMA–beta, insulinogenic index or disposition index) neither at the base line visit nor at the last visit.

Conclusion: Genetic variation in the *HNF-4α* P2 promoter significantly increases risk of future T2D as determined by two large prospective cohorts from Finland and Sweden.

0331

Common variation in the *LMNA* gene increases susceptibility to type 2 diabetes

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Background and Aims: *LMNA* is a positional and biological candidate gene for type 2 diabetes (T2D). The gene is located in the replicated area of T2D linkage on chromosome 1q, while mutations in *LMNA* cause a form of monogenic insulin resistance and diabetes (familial partial lipodystrophy). We hypothesised that, as in other monogenic causes of diabetes – such as those due to mutations in *PPARG*, *HNF4A* and *KCNJ11*- common variation in *LMNA* might predispose to multifactorial T2D.

Materials and Methods: We analysed 6 tagging SNPS (rs12063564, rs6661281, rs955383, rs693671, rs505058 and rs4641) in cases of T2D (n=2158) and non-diabetic controls (n=2574), all of UK origin. SNPs were selected from HapMap and previously published data, capturing>90% of common variation in the selected region at an r^2 of 0.8.

Results: Association with diabetes was seen with the rare allele for 3 SNPs which was strongest using a recessive model: rs12063564 (5′ upstream, MAF 0.17) OR 1.83 (95% CI 1.23–2.7), p=0.002; rs6661281 (5′ upstream, MAF 0.41) OR 1.19 (1.02–1.40), p=0.03; rs505058 (synonymous SNP exon 7, MAF 0.06) OR 3.68 (1.3–12.95), p=0.01.

Haplotype analysis did not reveal any additional association compared to the single point analysis.

In the diabetic cases no association was seen between any SNP and age at diagnosis of diabetes, BMI or waist–hip ratio.

Conclusion: In this first large case-control study, our results suggest that common variation in the *LMNA* gene affects susceptibility to T2D. This finding requires confirmation in further cohorts but is consistent with other examples of overlap between genes involved in both monogenic causation and multifactorial susceptibility.

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0332

Does the Val262Leu polymorphism in the mitochondial rhomboid protease *PSARL* predispose to type 2 diabetes in South Asians?

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Background and Aims: The genes encoding mitochondrial proteins involved in beta-oxidation of fatty acids are potential susceptibility genes for type 2 diabetes. One such candidate is the gene encoding the mitochondrial intramembrane protease presenilins-associated rhomboid-like protein (PSARL). *PSARL* is located on chromosome 3q27 in a region with substantial evidence for linkage with type 2 diabetes and metabolic syndrome phenotypes. Expression of the PSARL gene in human skeletal muscle is associated with insulin sensitivity and mitochondrial oxidative capacity. The common G→C SNP in exon 7 of the *PSARL* gene results in a change from valine to leucine at amino acid 262 in the encoded protein. A study of a population of northern European ancestry demonstrated that individuals homozygous for the G allele had higher plasma insulin concen-

trations compared with individuals with the GC or CC genotypes. The association of the *PSARL* genotype with type 2 diabetes was not explored, however. We present data from a pilot study which aimed to determine whether the G→C SNP of the *PSARL* gene is associated with type 2 diabetes in a distinct South Asian ethnic group.

Materials and Methods: Genotyping was performed in subjects recruited to the United Kingdom Asian Diabetes Study. All the individuals were from an ethnically homogeneous population, originating from north Pakistan and Azad Kashmir. This population is known to have a high prevalence of insulin resistance.

Results: 631 patients with type 2 diabetes and 250 ethnically-matched normoglycaemic control subjects were genotyped. The frequency of the less common C allele was 0.449 in the diabetic group compared to 0.452 in the control group (p=NS). There was no significant difference in the genotype frequencies between the groups. In subjects above the age of 50 years, however, the GG genotype was significantly more frequent in the control population compared to the diabetic group ($p<0.01$).

Conclusion: This pilot study had 81% power to detect a disease association with an odds ratio of 1.35 or above. The data suggest, therefore, that the G→C SNP in exon 7 of the PSARL gene is unlikely to be a major susceptibility determinant for type 2 diabetes, although we cannot exclude a minor role. The GG genotype of the *PSARL* gene may be protective against type 2 diabetes in older individuals, although this requires confirmation in a larger data set. Further studies investigating the effect of this polymorphism on beta-cell function and the development of insulin resistance are required.

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0333

Adenylyl Cyclase 3 (AC3) and the genetic susceptibility to type 2 diabetes in Swedish Caucasians

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Background and Aims: Goto–Kakizaki (GK) rat is an animal model of non-obese type 2 diabetes (T2D). A previous study from our laboratory demonstrated that the adenylyl cyclase 3 (AC3) gene is over-expressed in the pancreatic islets of the GK rat due to two point mutations in the promoter region. In the present study, we explore whether AC3 genetic variation confers susceptibility to T2D in men.

Materials and Methods: We first screened for mutation in the promoter region and then studied the single nucleotide polymorphisms (SNPs) covering the whole genomic region of the human AC3 gene. Genotyping experiments for 15 valid SNPs, including a novel identified variant -17A/T in the promoter, were performed in 294 patients with T2D, 319 subjects with impaired glucose tolerance (IGT) and 317 non-diabetic healthy individuals. All patients and subjects studied were Swedes.

Results: The single marker association analysis indicated that four SNPs, including rs2033655, rs1968482, rs934611 and rs1044040 were significantly associated with T2D. Further quantitative trait analysis related to SNP rs934611 revealed that T2D patients with GG genotype as compared with those carrying GA and AA genotypes had increased BMI (30.2±5.1 vs 28.3±5.6, P=0.032) and WHR (0.96±0.08 vs 0.90±0.05, P=0.041).

Conclusion: We demonstrate that AC3 genetic variations confer the susceptibility to the development of T2D among Swedish Caucasians.

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0334

Gene-gene interaction between the type 2 deiodinase gene (*DIO2*) Thr92Ala variant and the β2 adrenergic receptor gene (*ADRB2*) Arg16Gly variant is associated with elevated glycaemia in the general population

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Background and Aims: The *DIO2* gene encodes the type 2 deiodinase (D2), which is activated by the β-adrenergic pathway. D2 catalyzes the conversion of thyroxine (T_4) to the active form of thyroid hormone (T_3). T_3 is an important factor in energy metabolism by its ability to stimulate the expression of genes involved in thermogenesis and regulation of glucose metabolism in skeletal muscle and adipose

tissue. The Thr92Ala polymorphism (rs225014) in *DIO2* has previously been associated with insulin resistance in women and diabetic patients. The aim of this study was to investigate the possible association between the *DIO2* Thr92Ala polymorphism and type 2 diabetes (T2D), obesity, and related metabolic quantitative traits including measures of insulin resistance in 7,348 middle-aged Danish whites. We also hypothesized that variation in β-adrenergic receptor genes contributes to association with metabolic traits via interactions with *DIO2*.

Materials and Methods: The *DIO2* Thr92Ala polymorphism was genotyped in the large population-based Inter99 cohort of 5,942 subjects and in a group of 1,406 type 2 diabetic patients.

Results: No significant association between either T2D or obesity and the *DIO2* Thr92Ala polymorphism was found. Neither, did we find association between the genotype and obesity- and T2D related quantitative metabolic traits, including fasting and post-OGTT levels of plasma glucose and serum insulin, and measures of obesity. However, we did observe a significant gene–gene interaction of the Thr92Ala *DIO2* variant and the Arg16Gly variant in the gene encoding the β2 adrenergic receptor (*ADRB2*) on fasting plasma glucose in a double recessive model (Ala92/Arg16 double homozygous (n=100): mean±SE 5.8 mmol/l±0.08 vs. all other genotypes: 5.5±0.01; epistatic model p=0.004, global model p=0.005). A possible mechanism for this interaction is through β-adrenergic receptor-D2-thyroid hormone stimulation of GLUT4 expression.

Conclusion: Middle-aged subjects homozygous for both the *DIO2* Ala-allele and the *ADRB2* Arg-allele have elevated fasting plasma glucose level. Further large-scale studies are needed to substantiate this finding.

0335

ATF6, a key regulator of the unfolded protein response, is associated with type 2 diabetes

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Background and Aims: Activating Transcription Factor 6 (*ATF6*) is critical for initiation and full activation of the unfolded protein response. We investigated *ATF6* for its association with type 2 diabetes in the Dutch population. An association between variations in *ATF6* and type 2 diabetes was recently reported in Pima Indians, but additional studies in more outbred study samples are strongly required to evaluate its significance.

Materials and Methods: Two independent Dutch cohorts, comprising in total 949 subjects, were used to investigate the genetic association between polymorphisms in the *ATF6*-gene and type 2 diabetes. Four SNPs with a high potential to affect the ATF6 protein levels/activity were genotyped in 152 subjects from the general population and analysed for their association with glucose, insulin and HOMA-index: SNP -116C/T in the promotor, an A/G Met67Val substitution in exon 3, a C/G Pro145Ala substitution in exon 5, and+124G/A in the 3′untranslated region of the *ATF6* gene. Significantly associatiated polymorphisms in the first cohort were further investigated for their contribution to type 2 diabetes in a second Dutch case-control cohort (N=797), that consisted of 210 type 2 diabetes patients, 208 IFG/IGT subjects and 379 normoglycaemic controls

Results: In subjects from the general population, the +124G-allele was associated with increased glucose levels (p=0.02), and HOMA-index (p=0.05). Furthermore, in the Dutch case-control cohort, the+124G-allele was more prevalent in subjects with IFG, IGT or type 2 diabetes, than in healthy individuals (49% versus 40%, OR=1.4, 95% confidence interval 1.09–1.91, p=0.011). This effect remained significant after adjustment for age, sex and body mass index (p=0.016).

Conclusion: These results confirm the recently reported association of *ATF6* with type 2 diabetes in Pima Indians, and highlight the potential importance of *ATF6* for conferring genetic susceptibility to type 2 diabetes in more outbred populations.

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0336

Gene polymorphisms of antioxidant enzymes in diabetes mellitus

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Background and Aims: Reactive oxygen species generated by hyperglycemia modify structure and function of lipids, proteins and other molecules taking part in chronic vascular changes in diabetes. Low activity of scavenger

enzymes has been observed in diabetic patients and their protective role in oxidative stress may be deteriorated. This study was undertaken to investigate the association between polymorphisms of selected genes of antioxidant enzymes and diabetic angiopathy.

Materials and Methods: Total of 220 patients with Type 2 diabetes (T2DM), 66 with Type 1 diabetes (T1DM) and control group of 110 nondiabetic healthy subjects unrelated to diabetic patients were examined. Microangiopathy was present in 159 patients, 35 subjects had macrovascular complications and 92 patients were free of any complications. Venous blood samples were drawn after an overnight fast. Paraoxonase (PON1) and superoxide dismutase (SOD) activities were determined spectrophotometrically. Genomic DNA was obtained by automated isolator. Polymorphisms were estimated by PCR-RFLP.

Results: The allele frequencies of PON1, SOD1, SOD2 and CAT are shown in Tab.1. Significant diferences between T1DM,T2DM and control persons (p<0,01) were found in coding region of PON1 and SOD2 genes (in table expressed by bold letters) but not in SOD1 and CAT genes. Serum PON1 activity was significantly decreased in T1DM (110±68 nmol/ml/min) and T2DM subjects (118±69 nmol/ml/min) compared to the control persons (203±58 nmol/ml/min). L55M and Q192R polymorphisms effected PON1 activity. Serum PON1 activity was higher in LL than in MM genotype (p<0,05) and similarly the presence of RR genotype was associated with higher PON1 activity than of QQ genotype (p<0,05) in both Types of diabetes. No associations were observed between SOD gene polymorphisms and SOD activity. Better diabetes control was found in patients with LL (HbA1c=7,4±0,2) than with MM (HbA1c=7,6±0,1; p<0,05) genotype of PON1. Significantly different allele frequencies were found in diabetic patients with macroangiopathy (M=0,59, R=0,11) than in those without (M=0,44, R=0,18; p<0,01) but no difference was associated with/without microangiopathy.

Tab1. The Allele frequencies.

	PON1 L55M	PON1 Q192R	PON1 -107C/T	PON1 -907G/C	SOD1 35A/C	SOD2 Ala-9Val	CAT -262T/A
T1DM	**L=0,521**	**L=0,813**	C=0,515	G=0,550	A=0,90	**A=0,480**	T=0,57
	M=0,479	**M=0,187**	T=0,485	C=0,450	C=0,10	**V=0,520**	A=0,43
T2DM	**L=0,583**	**Q=0,849**	C=0,520	G=0,553	A=0,91	**A=0,475**	T=0,58
	M=0,417	**R=0,151**	T=0,480	C=0,447	C=0,09	**V=0,525**	A=0,42
Controls	**L=0,690**	**Q=0,543**	C=0,555	G=0,590	A=0,92	**A=0,300**	T=0,57
	M=0,310	**R=0,457**	T=0,445	C=0,410	C=0,08	**C=0,700**	A=0,43

Conclusion: The results of our study demonstrate that oxidative stress in diabetes mellitus can be accelerated nor only due to increased production of reactive oxygen species by hyperglycemia but also by reduced ability of antioxidant defense caused at least partly by genetic polymorphisms of some scavenger enzymes. Relationship was found between PON1 polymorphisms and macroangiopathy in diabetic patients.

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PS 9

PPAR and adiponectin genes

0337

Genetic and non-genetic regulation of *PPAR*δ mRNA expression in human skeletal muscle

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Background and Aims: Peroxisome proliferator activated receptors (PPARs) are nuclear receptors regulating the expression of genes involved in lipid and glucose metabolism in a subtype- and tissue-specific manner. There are three different PPAR isoforms named α, δ and γ. In mice, PPARδ seems to be implicated in the regulation of fatty acid burning capacities of skeletal muscle and adipose tissue by controlling the expression of genes involved in β-oxidation and energy uncoupling. Our aim was to study the influence of age and insulin on *PPAR*δ mRNA expression in human skeletal muscle, and investigate whether *PPAR*δ mRNA expression is an inherited trait.

Materials and Methods: Muscle biopsies were obtained before and after hyperinsulinemic euglycemic clamps from 102 young (age 28±2 years, 62 monozygotic and 40 dizygotic) and 74 elderly twins (age 62±2 years, 36 monozygotic and 38 dizygotic). Body composition was determined by dual-energy x-ray absorptiometry. *PPAR*δ mRNA levels were quantified by real-time PCR using the ABI PRISM 7900 Sequence Detection System. *Cyclophilin A* was used as an endogenous control. Genetic modelling to estimate the degree of genetic vs. environmental influence on *PPAR*δ mRNA expression was performed separately in the two age groups using standard Mx scripts. Six SNPs were chosen for genotyping after analyzing HapMap data using the Tagger program. The *PPAR*δ SNPs (rs7744392, rs9470001, rs2267665, rs2076169, rs2076168 and rs1053046) were genotyped using allelic discrimination in the ABI PRISM 7900 Sequence Detection System.

Results: Young twins had significantly increased *PPAR*δ mRNA levels compared to elderly twins both in the basal state (2.5±0.18 vs. 1.3±0.08, *P*<0.000001) and after the 2-hour hyperinsulinemic euglycemic clamp (2.6±0.30 vs.

1.3±0.08, $P<0.000001$). The insulin clamp had no significant effect on *PPAR*δ mRNA levels neither in all individuals (2.0±0.12 vs. 2.1±0.19, $P=0.4$) nor in the young ($P=0.4$) or elderly twins ($P=0.8$) when analyzed separately. Biometric models were calculated for basal *PPAR*δ mRNA expression to estimate the degree of genetic vs. environmental influence. In both young (a^2 [additive genetic]=0.59, e^2 [unique environment]=0.41) and elderly twins ($a^2=0.63$, $e^2=0.37$) there was a major genetic component influencing basal *PPAR*δ mRNA levels. Young carriers of the rare allele at SNP rs1053046 displayed a trend towards higher *PPAR*δ mRNA levels compared to non-carriers in the basal state (2.9±0.40 vs. 2.4±0.19, $P=0.08$) and significantly higher levels after the hyperinsulinemic euglycemic clamp (4.8±2.0 vs 2.4±0.28, $P=0.02$). No such difference was observed among the elderly twins (0.9±0.17 vs. 1.3±0.08, $P=0.14$ in the basal state and 0.9±0.07 vs. 1.3±0.07, $P=0.39$ after the insulin clamp). No genotype-specific differences in *PPAR*δ mRNA expression were observed for any of the other polymorphisms. Regression analysis revealed that age ($P<0.0001$), central adiposity ($P=0.004$) and birth weight ($P=0.03$) were the best predictors of basal *PPAR*δ mRNA expression. Age ($P=0.002$) was the factor that best explained *PPAR*δ mRNA levels after insulin clamp.

Conclusion: We provide evidence that mRNA expression of *PPAR*δ in human skeletal muscle is reduced by age and under genetic control.

0338

Studies of association of the *PPARα* Leu162Val polymorphism in relation to obesity,dyslipidaemia, type 2 diabetes, and related quantitative traits

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Background and Aims: Peroxisome proliferator-activated receptor-α (PPARα) is a nuclear receptor capable of regulating proteins involved in lipid metabolism and glucose homeostasis in the liver and in skeletal muscle. PPARα regulates the expression of genes involved in peroxisomal and mitochondrial β-oxidation pathways, fatty acid uptake, and the catabolism of triglycerides. Previous studies of the Leu162Val polymorphism in PPARα have indicated association with obesity, type 2 diabetes, and dyslipidaemia, although the results have been somewhat discrepant.

Materials and Methods: The Leu162Val polymorphism was genotyped in a population-based sample (Inter99) of 6,174 Danish Caucasians (2,955 men, 2,986 women, age 46±8 years (mean±SD), BMI 26.3±4.6 kg/m^2) and 1,037 type 2 diabetic patients sampled at Steno Diabetes Center (625 men, 412 women, age 58.9±11 years, BMI 29.4±5.1 kg/m^2). All participants of Inter99 underwent a standard 75 g oral glucose tolerance test. Genotyping was performed using chip-based matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry.

Results: Using a general linear model with correction for sex, age, and BMI we found no association between the Leu162Val polymorphism and obesity-related quantitative traits (BMI, fasting serum triglyceride, fasting serum total and HDL cholesterol, and waist-to-hip ratio). In a case-control study no significant differences in allele frequency or genotype distribution between 1,383 type 2 diabetic patients and 4,401 healthy control subjects were observed. In addition, the Leu162Val polymorphism was not associated with dyslipidaemia in a case-control study comprising 1,399 dyslipidaemic subjects and 4,399 normolipidaemic subjects. Furthermore, we were not able to show any interaction between the Leu162Val polymorphism and SNPs in other PPAR isotypes (PPARδ, PPARγ) on serum triglyceride levels. Interestingly, for the Val162Val genotype an increase in fasting serum triglyceride concentration was observed (Leu/Leu+Leu/Val $n=5,777$, 1.33±1.35 mmol/l vs. Val/Val $n=17$, 2.22±2.4 mmol/l, $p=0.007$).

Conclusion: The PPARα Leu162Val polymorphism is not a major contributor in the pathogenesis of obesity, type 2 diabetes, dyslipidaemia, or related quantitative traits in a Danish Caucasian population-based cohort.

0339

Common polymorphisms of the PPAR-γ2, PGC-1α and adiponectin genes interact in their effects on the conversion from impaired glucose tolerance to type 2 diabetes: the STOP-NIDDM trial

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Background and Aims: Peroxisome proliferator-activated receptor (PPAR) γ is a nuclear hormone receptor that plays a role in adipocyte differentiation, particularly when co-activated by PPAR-γ coactivator 1α (PGC-1α), and modulates the production and release of adipose-derived factors including adiponectin, leptin, plasminogen activator inhib-

itor 1 (PAI-1), tumor necrosis factor α (TNF-α), and interleukin 6 (IL-6). The Pro12Ala polymorphism of the PPAR-γ2 gene (*PPARG2*) was the first genetic variant identified consistently associated with the risk of type 2 diabetes. Single nucleotide polymorphisms (SNPs) of the PGC-1α gene (*PGC1A*), and genes regulating adipokines have also been associated with diabetes. The aim of the present study was to investigate whether or not there is an interaction between SNPs of *PPARG2*, *PGC1A* and SNPs of genes encoding different adipokines in their effects on the conversion from impaired glucose tolerance to type 2 diabetes in subjects from the Study To Prevent Non Insulin Depending Diabetes Mellitus (STOP-NIDDM).

Materials and Methods: The STOP-NIDDM trial evaluated the effect of acarbose compared to placebo on the prevention of type 2 diabetes. The following SNPs were genotyped in 768 DNA samples available: Pro12Ala of *PPARG2*, Gly482Ser of *PGC1A*, SNPs+45 in exon 2 and +276 in intron 2 of the adiponectin gene, G-308A in the promoter of the TNF-α gene, C-174G in the promoter of the IL-6 gene, pentanucleotide insertion/deletion polymorphism of the leptin receptor gene and -675 4G/5G polymorphism in the promoter of the PAI-1 gene.

Results: The Pro12Ala of *PPARG2* and Gly482Ser of the *PGC1A* had a significant interaction with the haplotype of the two SNPs of the adiponectin gene with respect to the conversion to diabetes (*P* values for the interaction 0.005 and 0.036, respectively). Subjects carrying the three risk genotypes of SNP+45 and SNP+276 of the adiponectin gene and the Pro12Ala polymorphism of *PPARG2* had a 2.82-fold higher risk for the development of diabetes (95% confidence interval (CI) 1.28–6.23, *P*=0.010) compared to those who did not carry these risk alleles. In the placebo group, carriers of the previously mentioned risk genotypes had a 5.71-fold increase in the risk of diabetes (95% CI 1.57–20.78; *P*=0.008). The presence of the three risk genotypes of the Gly482Ser polymorphism of *PGC1A* and both SNPs of the adiponectin gene resulted in 3.42-fold increase in the risk of diabetes (95% CI 1.44–8.14, *P*=0.005) compared to subjects who did not have the risk genotypes. In the placebo group the corresponding odds ratio (OR) was 13.26 (95% CI 2.65–66.28, *P*=0.002). The ORs for the conversion to diabetes associated with the four risk alleles (SNPs of *PPARG2*, *PGC1A* and the adiponectin gene) was not higher than that of the three risk alleles reported above. *P* values for the interaction between the polymorphisms of *PPARG2*, *PGC-1A* and SNPs of TNF-α, IL-6, leptin receptor and PAI-1genes with respect to the conversion to type 2 diabetes were not statistically significant, and the effect of the combined genotypes on the risk of diabetes was not increased beyond that of individual SNPs.

Conclusion: Our results suggest that SNPs in the *PPARG2*, *PGC-1A* and adiponectin genes may interact in their effects on the risk for type 2 diabetes.

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0340

The protective effect on the risk of type 2 diabetes given by the PPARγ2 Ala12 variant is heterogeneous across different populations

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The P12A SNP of PPARγ2 gene has been studied for association with type 2 diabetes (T2D) with controversial results, thus suggesting variability in its contribution to disease risk. To deeper investigate this issue, a meta-analysis of all 39 published association studies (i.e. PubMed search using "PPARγ and diabetes", "genetics of type 2 diabetes" and "candidate gene" as key-words) and two unpublished Italian studies for a total of 41,296 individuals was performed.

In the 2 Italian studies, Caucasians from Gargano (n=1183) or Calabria (n=1196) were recruited. The 2 studies were approved by local Ethical committees. The P12A SNP was screened by dideoxy single-base extension of an unlabeled primer (ABI PRISM SNaPshot Multiplex Kit 4323151).

Risks are expressed As OR and 95% CI. Between-studies heterogeneity was assessed with Cochran's Q test. Random effects meta-analysis and meta-regression were used to estimate overall ORs and to explore heterogeneity. Permutation resampling p-values were calculated to address the risk of spurious significant results. All the analyses were performed using SAS (Release 9.1, Cary, NC, USA, 2002–2003).

In all 41 studies the P12A SNP was in Hardy-Weinberg equilibrium. In random-effects meta-analysis of data from all 41,296 individuals, A12 carriers showed a 19% risk reduction of T2D (OR=0.81, 95% C.I.=0.75–0.88); this effect was heterogeneous across all studies (p=0.004). Data were then stratified according to the 3 regions where studies had been conducted: Asia, Europe and North-America. The

risk reduction given by the A12 variant was significantly different (ORs heterogeneity p=0.05), being 35% among Asians and 15% among both Europeans and North Americans. Risk reduction in Asia was different from that in Europe (p=0.02) but not from that in North America (p=0.09), very likely because of the small size of the latter sample. When data from Europe were stratified according to a North to South gradient, the risk reduction of T2D in Ala12 carriers was progressively reduced (test for trend in the ORs p=0.02) from North (25%, p<0.0001) to Centre (10%, p=0.04) and South (0%, p=0.94) Europe. The same trend was significant even when assessed via permutation re-sampling (p=0.041). Our meta-analysis on more than 41,000 individuals shows that the A12 variant confers a reduced risk of T2D. This effect is not homogeneous, being greater in Asians than in other populations, and among Caucasians from Europe, being stronger in Northern, lower and barely significant in Central and null in Southern Europe. To the best of our knowledge this is the first study which clearly indicates heterogeneity of the genetic risk of T2D and stress, therefore, the need of great caution when trying to interpret and reconcile data obtained in populations from different geographical regions and/or of different ethnicity.

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0341

Haplotype structure and association testing of the peroxisome proliferator-activated receptor δ *PPARD* with insulin resistance in a population-based sample 6,125 middle-aged subjects

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Background and Aims: Studies in animals reveal that peroxisome proliferator-activated receptor δ (PPARδ) regulates glucose metabolism and insulin sensitivity both in the liver and skeletal muscles. Moreover, PPARδ enhances fatty acid catabolism and in skeletal muscle PPARδ additionally augments physical endurance, increases the proportion of slow twitch type I muscle fibres and increases oxidative metabolism thereby averting obesity. Thus, we hypothesized that variation in *PPARD* associates with insulin resistance and this impact is accentuated by interaction with known variants in *PPAR* and *PPARGC1* genes reported to increase the risk of insulin resistant traits.
Materials and Methods: We studied variation in the coding region of *PPARD* in 64 type 2 diabetic patients and 24 healthy subjects selected for high or low physical fitness. Based upon the results of this variant detection and information derived from the HapMap data resource common variants and haplotype tagging single nucleotide polymorphisms (SNPs) selected using the Tagger approach were genotyped in the population-based Inter99 cohort of Danish whites (n=6,125) using TaqMan allelic discrimination.
Results: Fifteen nucleotide variants were identified; four with an estimated minor allele frequency above 5%. A total of twelve SNPs including haplotype tagging variants were genotyped. In the population-based Inter99 sample we studied quantitative metabolic traits and observed a significant association of rs2076167 and haplotypes including this variant and rs6902123 with measures of insulin resistance (fasting serum insulin, p<0.001; HOMA insulin resistance, p=0.001; and post-OGTT incremental area under the insulin curve, p=0.03) when evaluating subjects with treatment-naïve impaired glucose regulation (IFG, IGT, or screen-detected type 2 diabetes, n=1,393). When examining subjects with normal glucose tolerance (n=4,346) this effect was not evident. In addition, we tested for a potential epistatic effect of SNPs in *PPARD* and common variants in the *PPARG*, *PPARA*, *PPARGC1A*, and *PPARGC1B* genes on measures of insulin resistance. No epistatic effects were, however, identified.
Conclusion: Exhaustive evaluation of variation in *PPARD* reveals that SNPs and haplotypes of *PPARD* associate with measures of insulin resistance in subjects with treatment-naïve impaired glucose regulation. These results suggest that variation in *PPARD* may influence insulin resistance at a critical and vulnerable time point in the pathogenesis of type 2 diabetes at which subjects are further strained by environmental and genetic risk modulators.

0342

Association between the C-11377G promoter variant of the *ACDC* gene and carotid intima thickness in healthy subjects

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Background and Aims: The *ACDC* gene encodes the protein adiponectin and lower adiponectin levels have been reported in patients with type 2 diabetes and coronary artery

disease. Common variants in the promoter region of the *ACDC* gene have been associated with adiponectin levels and type 2 diabetes. The purpose of this study was to examine the relationships between common variants in the *ACDC* promoter region and carotid intima-media thickness, a marker of cardiovascular disease, in a cohort of healthy subjects participating in the RISC study (Relationship between Insulin Sensitivity and Cardiovascular disease study).

Materials and Methods: Healthy subjects aged 30–60 yrs were recruited at 19 centres in 14 European countries. B-mode ultrasound images of the common carotid artery were recorded by a single operator at each centre, and intima media thickness (cIMT) was measured in digitized end-diastolic frames in the central laboratory by a single reader. SNPs A-11426G, G-11391A and C-11377G were genotyped using the TaqMan system (Applied Biosystems, Warrington, UK). Haplotypes were inferred using the PHASE program. Fasting adiponectin levels were normalised by log transformation and the geometric means are presented.

Results: The study cohort consists of 1325 subjects (597 men and 728 women) aged 43.8±8.3 yrs (mean±SD), with a mean BMI of 25.5±4.1 kg/m^2. All SNPs were in Hardy-Weinberg equilibrium and common (minor allele frequency>0.05). Of the 3 SNPs, only C-11377G was significantly associated with cIMT. Carriers of the G allele had significantly greater cIMT values compared to C allele homozygotes (mean±SE, 608±3.9 vs. 596±3.3 μm, p=0.017). This difference remained after adjusting for age, sex, recruitment centre and BMI (p=0.02), and after inclusion of fasting adiponectin levels to these covariates (p=0.025). The haplotype pair (diplotype) containing the alleles -11426A, -11391G and -11377G was significantly associated with higher cIMT values compared to all other diplotypes (610±4.8 vs. 595±3.4 μm, p=0.006). This difference remained significant after accounting for the same factors and covariates.

Conclusion: Variation at SNP C-11377G in the *ACDC* gene promoter is associated with increased cIMT in healthy subjects, and is independent of circulating adiponectin levels.

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0343

Studies of polymorphisms of the adiponectin encoding gene *ADIPOQ* show an association of a -11391G allele with insulin resistance in the general middle-aged population of whites

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Background and Aims: The *ADIPOQ* gene at chromosome 3q27 encoding adiponectin, which plays an important role in glucose and lipid metabolism, is a positional and biological candidate gene for type 2 diabetes and the metabolic syndrome. Seven polymorphisms in the *ADIPOQ* gene (a -11426A>G, a -11391G>A, a -11377C>G, a -4041A>C, a+45T>G, a+276G>T, and a+2019delA) have been reported to associate with type 2 diabetes, insulin resistance and/or circulating adiponectin levels. The aim of the present study was to examine the potential effect of these seven polymorphisms on susceptibility of type 2 diabetes, the metabolic syndrome, and related phenotypes in a large-scale study of Danish whites.

Materials and Methods: Seven polymorphisms of the *ADIPOQ* gene were genotyped in the population-based Inter99 study cohort (n=6,514) and in a group of type 2 diabetic patients (n=1098) by analysis of PCR-generated primer extension products using high-throughput chip-based matrix-assisted laser desorption/ionisation time-of-flight mass spectronomy.

Results: No association of these variants with type 2 diabetes or the WHO-defined metabolic syndrome were demonstrated. A meta-analysis including previously published case-control studies of the examined SNPs and our own results did not revealed any association with type 2 diabetes. However, we found an association of the -11391G allele with higher fasting serum insulin levels (p=0.005) and higher HOMA insulin resistance index (p=0.007) in a population based sample of 4,432 middle-aged glucose tolerant subjects.

Conclusion: Our data suggest that the -11391G>A polymorphism of the *ADIPOQ* gene may contribute to the inter-individual variation of serum insulin and insulin sensitivity, whereas the examined polymorphisms of the *ADIPOQ* are not directly associated with type 2 diabetes or the metabolic syndrome in the Danish Caucasians.

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0344

The A-11426G and G-11391A promoter variants of the *ACDC* gene influence adiponectin levels in healthy subjects

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Background and Aims: The *ACDC* gene encodes the protein adiponectin and decreased circulating adiponectin levels have been observed in insulin resistance and type 2 diabetes. Single nucleotide polymorphisms (SNPs) in the promoter region of the *ACDC* gene have been associated with adiponectin levels and type 2 diabetes**.** We investigated the role of the *ACDC* SNPs A-11426G, G-11391A, C-11377G and their haplotypes with adiponectin levels and insulin sensitivity in a cohort of healthy subjects participating in the RISC study (Relationship between Insulin Sensitivity and Cardiovascular disease study).
Materials and Methods: Healthy subjects aged 30–60 yrs were recruited at 19 centres in 14 European countries. Each subject underwent anthropometric and metabolic assessment (standard OGTT), and whole body insulin sensitivity (M value) was assessed by euglycaemic hyperinsulinaemic (40 mU/m^2/min) clamp. SNPs were genotyped using the TaqMan system (Applied Biosystems, Warrington, UK). Haplotypes were inferred using the PHASE program. Fasting plasma adiponectin levels were normalised by log transformation and the geometric means are presented.
Results: The study cohort consists of 1325 subjects (597 men and 728 women) aged 43.8±8.3 yrs (mean±SD), with a mean BMI of 25.5±4.1 kg/m^2. All SNPs were in Hardy–Weinberg equilibrium and common (minor allele frequency>0.05). Fasting plasma adiponectin was significantly correlated with insulin sensitivity (r=0.33, p<0.001) and inversely correlated with fasting serum insulin (−0.34, p<0.001). Of the 3 SNPs, A-11426G and G-11391A were associated with fasting plasma adiponectin levels. For SNP A-11426G, subjects homozygous for the A allele had significantly higher plasma adiponectin levels compared to G allele carriers (geometric mean (interquartile range) 7.7 (5.6–10.5) vs. 7.2 (5.4–9.6) mg/l, p=0.015). This relationship remained significant after adjusting for age, sex, recruitment centre and BMI (p=0.008). Carriers of the A allele of SNP -11391 had significantly higher plasma adiponectin levels compared to the G/G genotype (9.1 (6.9–12.2) vs. 7.4 (5.4–9.7) mg/l, p<0.0001) and remained significant after adjusting for the same factors and covariates (p<0.0001). The haplotype pair (diplotype) containing the alleles -11426G, -11391G and -11377C was significantly associated with lower adiponectin levels when compared to all other diplotypes (6.8 (5.2–8.7) vs. 7.6 (5.6–10.3) mg/l, p=0.008). The difference remained significant after accounting for the same factors and covariates. However, there were no associations between the A-11426G and G-11391A SNPs (individually or as diplotypes) with the M value and fasting insulin levels, before and after correction for the key covariates.
Conclusion: We confirm that variation in the *ACDC* gene promoter at SNPs A-11426G and G-11391A influences circulating adiponectin levels, but not measures of insulin sensitivity.
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0345

The promoter region of adiponectin gene is a determinant in modulating insulin sensitivity in childhood obesity

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Background and Aims: Adiponectin (ACDC) is a recently discovered adipose tissue-specific cytokine that plays a role in modulating insulin sensitivity. Different single-nucleotide polymorphisms (SNPs) identified in the adiponectin gene, were found to be associated with the risk of diseases such as: obesity, insulin resistance, type 2 diabetes (T2DM) and metabolic disorders. The aim of our study was to assess the association of the -11391G>A, -11377C>G,+45T>G and+276G>T adiponectin SNPs and expected haplotypes with the metabolic abnormalities of insulin resistance in 270 overweight/obese children and to distinguish the effect of each SNP independently on the investigated phenotypes.
Materials and Methods: The SNPs were genotyped using the flurogenic 5′ nuclease assay application of the ABI PRISM 7900 HT Sequence Detection System. We performed ANOVA analysis to obtain initial overall p-values without assuming any specific genetic model. The effect of the polymorphisms on quantitative variables was investigated using multiple linear regression analysis. The values were adjusted for BMI-z, age and pubertal stage. Haplotype analysis was performed by using THESIAS software. Serum adiponectin levels were performed in 150 children and determined by radioimmunoassay (RIA Kit, LINCO Research Inc, USA).
Results: The GG genotype at the -11391 locus was associated with higher fasting insulin levels, HOMA-IR index and lower adiponectin levels compared to GA+AA genotypes (p=0.01, p=0.002 and p=0.03, respectively). Children heterozygous or homozygous for G allele at the -11377 locus showed higher fasting glucose (p=0.001 for both), fasting insulin (p=0.001 for both), HOMA-IR index (p<0.001 for both), triglyceride levels (0.02 and p=0.03, respectively) and lower adiponectin levels (p=0.002 and p=0.02, respectively) compared to C homozygous. The +45G carriers showed higher fasting and 2 h glucose levels (p=0.01 for both) and lower adiponectin levels (p=0.02) compared to non carriers. The relation between SNPs and insulin resistance phenotypes does not persist after adjust-

ing for adiponectin levels (p>0.05). The GGTG haplotype was associated with insulin resistance parameters (p<0.05) compared to all the other haplotypes. Moreover the haplotype analysis suggested that considering the same haplotypic background, each of the three polymorphisms exerted an independent effect on investigated variables. The promoter haplotype GG, was strongly associated with a significant decrease of adiponectin levels and with higher degree of insulin resistance (p=0.01 for both)

Conclusion: The -11391G>A, -11377C>G and+45T>G SNPs seems to be associated with insulin resistance syndrome in overweight/obese Italian children. These polymorphisms independently influence the investigated variables. However the effect of+45T>G SNP seems to be marginal compared to the promoter SNPs. A major finding of the present study is the association of the promoter SNPs haplotype with lower adiponectin levels and consecutively with insulin sensitivity. Thus the influence of the -11391G>A, -11377C>G and +45T>G SNPs and related haplotypes on insulin resistance may be fully explained by their correlation on adiponectin levels.

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0346

The -11377 C>G promoter variant of the adiponectin gene predicts vascular events in male coronary patients

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Background and Aims: Low serum levels of the adipocyte-derived peptide adiponectin are associated with obesity, type 2 diabetes, and cardiovascular disease. The -11377 C>G promoter variant of the adiponectin gene recently has been linked to decreased adiponectin levels. No prospective data demonstrating an increased cardiovascular risk in individuals with the G versus the C allele are available. We therefore aimed at investigating the impact of the -11377 C>G polymorphism on the incidence of vascular events in a high risk cohort of male coronary patients.

Materials and Methods: In a consecutive series of 402 men who had undergone coronary angiography for the evaluation of coronary artery disease, vascular events were recorded over 4.1±0.4 years.

Results: The prevalence rates of the -11377 CC, GC, and GG genotypes among our patients were 56.5%, 37.1% and 6.5%, respectively. At baseline, serum adiponectin levels decreased significantly from the CC over the GC to the GG genotype (p_{trend}=0.003), and the prevalence of significant coronary stenoses of at least 50% increased gradually from the CC over the GC to the GG genotype (63.9%, 73.2%, and 88.5%, respectively; p_{trend}=0.004). Prospectively, the presence of the G allele of the adiponectin gene -11377 C>G polymorphism strongly and significantly predicted future vascular events (adjusted hazard ratio=1.703 [1.074–2.699]; p=0.023). Adjusted hazard ratios were 1.582 [0.971–2.577] and 2.403 [1.108–5.214] for patients with the CG and GG genotypes when compared to patients with the CC genotype (p_{trend}=0.011).

Conclusion: Among male coronary patients the -11377 C>G promoter variant of the adiponectin gene is i) associated with decreased serum adiponectin levels, ii) correlated with an increased prevalence of significant coronary stenoses and iii) strongly predictive of future vascular events.

PS 10

Obesity genes

0347

Cannabinoid receptor 1 gene variation increases risk for obesity and type 2 diabetes and modulates phenotypes related to endocannabinoid-mediated physiological pathways

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Background and Aims: The endocannabinoid pathway contributes to the regulation of metabolism and food intake. To determine whether variation in the Cannabinoid Receptor gene (*CNR1*) modulates susceptibility to obesity, type 2 diabetes (T2DM) and related phenotypes we used large scale genetic epidemiology.

Materials and Methods: By sequencing all *CNR1* coding regions, exon–intron boundaries and regulatory regions, 68 Single Nucleotide Polymorphisms were identified, 36 had a

minor allele frequency (MAF) ≥5%. Twenty-one tagged polymorphisms, which summarize *CNR1* locus genetic variability were genotyped in French Caucasian obese adults and children (n=2,016), T2DM cases (n=1,519) and controls (n=1,299). The seven polymorphisms showing evidence of an association in more than one case/control study, underwent further familial analysis. Potential modulation of metabolic, fat mass, bone mass density and behavioural traits were then analyzed in 819 obese children, 865 Swiss obese adults, 5,065 middle-aged adults from a general French population (D.E.S.I.R.), and 1,474 postmenopausal Danish women.

Results: We identified five polymorphisms associated with both obesity (Odds ratios (ORs) ranging from 1.21 to 1.37; $p<0.03$ to<0.0006) and T2DM (ORs 1.21 to 1.28; $p<0.01$ to<0.008) and 4 with obesity only (ORs 1.17 to 1.36; $p<0.05$ to<0.00009). Quantitative trait analysis in these studies showed that the *CNR1* polymorphisms modulate food intake behaviour, smoking habits, alcohol consumption, fat mass, bone mass parameters, insulinaemia, glycaemia, and lipid and leptin levels.

Conclusion: Several *CNR1* polymorphisms increase the risk of obesity and T2DM and modulate body composition, glucose and lipid metabolisms and addictive behaviours, confirming a major role of the endocannabinoid system in the regulation of energy balance. This data from 12,238 European subjects provides physiological support for the use of cannabinoid receptor 1 antagonists for obesity and associated metabolic complications and further exploration of the endocannabinoid pathway using pharmaco-genomics is required.

0348

An exon4+665C>T polymorphism of the gene encoding Wingless-Type Mammary Tumor Virus Integration Site Family Member 3A (WNT3A) protects against obesity in a general population of whites

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Background and Aim: Evidence suggests that WNT-signaling and genes related to the WNT-signaling pathway may be important regulators for adipogenesis. The *WNT3A* gene encodes Wingless-Type Mammary Tumor Virus Integration Site Family Member 3A, which in recent studies has been shown to possess an inhibitory effect on adipogenesis. The aim of this study was to examine a known *WNT3A* gene polymorphism for an association with obesity and related quantitative metabolic traits.

Materials and Methods: One polymorphism (*WNT3A* exon 4+665 C>T, rs752107) was selected from the IMSJST (Institute of Medical Science-Japan Science and Technology Agency) Japanese SNP database for genotyping within the *WNT3A* gene locus. The *WNT3A* 665 C>T polymorphism was genotyped using the PCR-Invader technology in the population-based Inter99 study cohort of 5,846 middle-aged and treatment-naïve subjects. Within this cohort a case-control study for obesity was undertaken in 963 obese (BMI ≥30 kg/m^2) subjects and 2,588 lean, glucose-tolerant control participants (BMI <25 kg/m^2). Quantitative trait studies of metabolic variables were carried out in all 5,846 subjects of the Inter99 sample.

Results: The *WNT3A* 665 C>T T-allele frequency was *lower* in obese participants compared to control persons (30.9% [95% CI: 28.9–33.0] vs. 34.6% [95% CI: 33.3–35.9], P=0.003; Odds ratio: 0.73 [CI: 0.56–0.94]). The difference in genotype distribution was CC/CT/TT 47/44/9% (cases) vs. 42/46/12% (control), respectively (P=0.01). Furthermore, in the quantitative trait studies, there was a significant difference in BMI between the different genotype carriers (mean±SD: BMI CC: 26.36±4.55; CT: 26.12±4.46 and TT: 25.76±4.38 kg/m^2, P=0.002). There was no association between variation of the *WNT3A* gene and other quantitative anthropometric or metabolic traits.

Since it has been suggested that WNT signaling and genes related to the WNT signaling pathway are important regulators for adipogenesis we examined if there was a potential gene–gene interaction between the *WNT3A* 665 C>T polymorphism and the *PPARG* Pro12Ala polymorphism (rs1801282). Using a linear model we observed a significant epistatic effect (P=0.03) between the two polymorphisms. This epistatic effect was more marked (P=0.006) when assuming equal effect for Pro/Ala and Ala/Ala. The 2-loci genotype with protective alleles from both polymorphisms (n=159) had lower BMI (mean±SE: 24.7±0.6 kg/m^2) compared to the other genotypes (n=5,620) (26.2±0.1 kg/m^2, P=0.00007).

Conclusion: The minor T-allele of the *WNT3A* 665 C>T polymorphism may protect against obesity in middle-aged whites. Also, the *WNT3A* 665C>T polymorphism interacts with the *PPARG* Pro12Ala polymorphism to influence BMI.

0349

***GAD2* -243A> G polymorphism associates with measures of obesity and glycaemia and interacts with the *GCK* -30G>A polymorphism in a population-based sample of 5,857 middle-aged people**

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Background and Aims: *GAD2* encoding the glutamic acid decarboxylase enzyme GAD65 is located on chromosome 10p12, a region which has been linked to morbid obesity. A *GAD2* -243A>G polymorphism is reported to associate with morbid obesity among French people. The aim of the present study was in a population-based sample of middle-aged white Danes to investigate the relationship between the *GAD2* -243A>G polymorphism and estimates of obesity and glycaemia, and to examine for epistatic interactions with other genetic variants known to influence obesity and glycaemia.
Materials and Methods: The *GAD2* variant was genotyped in 5,857 treatment-naïve subjects from a population-based sample (Inter99) (2,920 men, 2,937 women, age 46±8 years (mean±SD), BMI 26.2±4.5 kg/m^2). All subjects underwent a standard 75 g OGTT. The *GAD2* -243A>G variant was genotyped using chip-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.
Results: The G-allele frequency among 2,582 subjects with a BMI of <25 kg/m^2 was 19.5% (95% CI 18.4–20.6) while among 968 subjects having BMI ≥30 kg/m^2 the frequency was 17.1% (15.5–18.8) (P=0.03), giving an odds ratio of 0.90 (0.74–0.98) for the difference in allele frequencies. Also, the genotype distributions were different between the groups: BMI <25 kg/m^2 AA 65%, AG 31%, GG 4%; BMI ≥30 kg/m^2 AA 69%, AG 29%, GG 3%, P=0.01. When analysing data in the total population of 5,857 treatment-naïve subjects GG carriers had lower fasting and post-OGTT plasma glucose: AA 5.6±0.8, AG 5.5±0.8, GG 5.5±0.8 mmol/l, P=0.008; 30-min post-OGTT AA 8.7±1.9, AG 8.6±1.9, GG 8.6±2.0 mmol/l, P=0.04. Similarly, in the subgroup of glucose-tolerant subjects (n=4,431) GG carriers had lower fasting plasma glucose concentrations: AA 5.3±0.4, AG 5.3±0.4, GG 5.2±0.4 mmol/l, $P=9\cdot10^{-5}$. No epistatic interactions between the *GAD2* variant and variants in genes reported to associate with obesity *(PPARGC1A, PPARGC1B, PPARG2,* and *NPY2R)* were observed. Since GAD65 is also expressed in pancreatic β-cells we examined for a potential interaction between the *GAD2* -243A>G polymorphism and several variants in the *TCF1A*, *HNF4A*, *KCNJ11*, and *GCK* genes with known impact on fasting plasma glucose. We observed a significant epistatic effect (P=0.002) and a significant global statistic ($P=6\cdot10^{-9}$) on fasting plasma glucose between a common *GCK* -30A>G variant and the *GAD2* -243A>G polymorphism. Subjects homozygous for the *GCK* A-allele and homozygous for the major *GAD2* A-allele had higher fasting plasma glucose concentrations: AA/AA 5.8±1.3 mmol/l versus all remaining genotype combinations 5.5±0.8 mmol/l, $P<10^{-5}$ when adjusted for sex, age, and BMI. Comparing glucose-tolerant subjects (n=4,387, AA/AA carriers=54) with hyperglycaemic subjects (IGT, IFG, and T2DM, n=1,531, AA/AA carriers=38) we found that subjects homozygous for the risk alleles of both polymorphisms had an estimated OR of 2.04 (1.31–3.16) for having hyperglycaemia (P=0.001).
Conclusion: The *GAD2* -243A>G polymorphism associates with obesity and fasting and post-OGTT glycaemia in a population of middle-aged whites. Moreover, the *GAD2* -243A>G polymorphism may interact with the *GCK* -30A>G variant to influence the fasting plasma glucose levels.

0350

***ENPP1* K121Q associates with severe obesity and T2D, and predicts overweight/class III obesity and familial glucose intolerance/T2D incidence in the prospective DESIR Study**

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Background and Aims: Variation in Ecto-Nucleotide Pyrophosphatase Phosphodiesterase 1 (ENPP1) gene associate with childhood and adult severe familial obesity and type 2 diabetes (T2D) in the French population. We analyzed the K121Q, IVS20delT-11 and A/G+1044TGA ENPP1 polymorphisms in a large French Caucasian cohort followed 9 years, D.E.S.I.R., to replicate previous results, and to assess the predictive value of ENPP1 polymorphisms on the risk of developing obesity and/or T2D in a French Caucasian general population.
Materials and Methods: We genotyped 5,153 middle-aged subjects (at baseline: men/women ratio: 49.6/50.4%; age: 47.2±10.0 y., body mass index (BMI): 24.7±3.8 kg/m^2). Genotyping used Taqman (Applied Biosystem) and Light Typer (Roche) technology. χ^2 test and P values were empirically computed with the CLUMP program.

Results: At baseline, the Q121Q genotype was associated with higher risk of class II obesity (BMI ≥35 kg/m^2; OR=3.37, p=0.01), class III obesity (BMI ≥40 kg/m^2; OR=6.23, p=0.009), T2D (OR=2.29, p=0.04), T2D with familial history (OR=5.80, p=0.00004), glucose intolerance (GI) or T2D with familial history of T2D (OR=2.89, p=0.003) when affected individuals were compared to the 1,245 lean normal glucose tolerant subjects (BMI <25 kg/m^2). Among normal weight participants at inclusion, the Q121 allele carriers had a trend towards an increased risk to develop overweight (BMI ≥27 kg/m^2; OR=1.18, p=0.06) and class III obesity (OR=2.14, p=0.07) during 9-year follow-up. In addition, 121Q allele increased the incidence of impaired glucose tolerance or frank T2D in context of familial T2D (OR=1.42, p=0.04). IVS20delT-11 and A/G+1044TGA polymorphisms didn't associate with any trait at baseline and during the follow-up, and the [Q-delT-G] haplotype did not show more significant results than the K121Q polymorphism analyzed alone.
Conclusion: We confirmed in a general French population the association of K121Q polymorphism with severe adult obesity and T2D. Our results also suggest a potential predictive value of K121Q on the risk of developing overweight/class III obesity and familial impaired glucose tolerance or frank T2D.
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0351

Large-scale studies of association between the functional *ADRB1* Arg389Gly polymorphism and obesity and hypertension

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Background and Aims: The β-adrenergic receptors are expressed in several tissues and are activated by catecholamines as part of the sympathetic nervous system. Activation of the β_1-adrenergic receptor in adipocytes and cardiac myocytes causes increased lipolysis and enhanced cardiac output, respectively. Variants in the gene encoding this cell surface receptor, β_1-adrenergic receptor (*ADRB1*), have therefore been hypothesised to be involved in the pathogenesis of both obesity and hypertension. The frequent and functional *ADRB1* Arg389Gly variant influencing the adenylyl cyclase activity of the receptor has with ambiguous results been examined for association with both obesity and hypertension. For clarifying the potential impact of this variant in relation to obesity and hypertension we examined the Arg389Gly variant in a relatively large-scale study sample of middle-aged subjects.
Materials and Methods: The *ADRB1* Arg389Gly polymorphism (rs1801253) was genotyped in a total of 7,677 Danish whites (3,886 men, 3,791 women) using a chip-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of PCR-generated primer extension products.
Results: A weak association between the codon 389 Gly-allele and obesity defined as BMI ≥30 kg/m^2 was observed comparing Gly-allele frequencies (non-obese n=6,108, 25.2% [24.4–25.9] vs. obese n=1,540, 26.9 [25.4–28.5], p=0.05) and genotype distribution (non-obese Arg/Arg 56%, Arg/Gly 37%, Gly/Gly 6% vs. obese Arg/Arg 53%, Arg/Gly 41%, Gly/Gly 7%, p=0.05). No association was observed in quantitative trait analyses of BMI, waist circumference, waist-to-hip ratio, or fasting serum lipid concentrations (triglyceride, total and HDL cholesterol). Moreover, no association was observed for WHO-defined hypertension; however, in quantitative trait analyses carriers of the Gly-allele had a slightly lower diastolic blood pressure (p=0.01), systolic blood pressure (p=0.02), and mean arterial blood pressure (p=0.008).
Conclusion: Despite the observed minor influence of the *ADRB1* Arg389Gly polymorphism on BMI and blood pressure our results suggest that this common gene variant *per se* does not have any major impact in the pathogenesis of obesity and hypertension in the examined population of Danish whites.

0352

Quantitative traits related to obesity: heritability, candidate genes and the EFPTS twins study

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Background and Aims: Both lifestyle factors and genetic predisposition play a role in the development of obesity. In the present study the contribution of genetic and environmental factors on the phenotypic variation of several obesity-related traits were determined by making use of the classical twin study approach. In order to explain (part of) the genetic variance, sib-pair linkage analyses were performed using microsatellite markers located near (<1.5 cM) five obesity-related genes, consecutively leptin (*LEP*), leptin receptor (*LEPR*), adiponectin (*APM1*), peroxisome proliferative activated receptor gamma (*PPARγ*) and resistin (*RETN*).
Materials and Methods: In the present study participated 480 monozygotic (MZ) and 276 dizygotic (DZ) healthy

twins (388 men, mean age 25.6) recruited from the East Flanders Prospective Twin Survey (EFPTS). All twins have undergone a clinical investigation where several obesity-related traits have been measured. Genetic (heritability) and environmental variance components were estimated using the structural equation modeling approach, which is implemented in the Mx package. Subsequently, variance-component linkage analyses were carried out using both the statistical programs Mx and Merlin. When appropriate, traits were adjusted for possible confounders including sex, (gestational) age, parity, height and adiposity.

Results: Heritabilities of body weight, body mass index (BMI), fat body weight, lean body weight, waist to hip ratio (WHR) and sum of four skin folds were high and ranged between 70 and 83%. Total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, total/HDL cholesterol ratio, leptin and free fatty acids concentrations had heritabilities varying from 37 to 79%. Birth weight had a low heritability of 24%. No sex differences in heritability estimates were observed. Marker D7S530 (*LEP*) showed suggestive linkage with BMI (Mx LOD=1.70; Merlin LOD=1.01), fat body weight (Mx LOD=1.33; Merlin LOD=1.54) and WHR (Mx LOD=1.39; Merlin LOD=1.33) and in Merlin with birth weight (LOD=1.57). Marker D3S1602 (*APM1*) showed suggestive linkage with HDL (Mx LOD=1.59; Merlin LOD=1.65) and leptin (Mx LOD=2.16; Merlin LOD=2.79) and in Mx with triglycerides concentrations (LOD=1.02). Finally, marker D3S3686 (*APM1*) showed suggestive linkage with triglycerides levels (Mx LOD=1.24; Merlin LOD=1.02).

Conclusion: This study illustrates that the genetic contribution to obesity-related intermediate traits is high. In addition, linkage analysis indicates that polymorphisms close to the *LEP* and *APM1* gene may be responsible for a part of this genetic contribution to several of these obesity-related intermediate traits.

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0353

Endothelial nitric oxide synthase (eNOS) E298D and T-786C gene polymorphisms do not affect post challenge insulin levels in non diabetic hypertensive men

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Background and Aims: The endothelial nitric oxide synthase (eNOS) gene has been suggested to modulate nitric oxide production and to regulate muscle blood flow by inducing vasodilation. Throughout this mechanism eNOS may play an important role in the regulation of insulin sensitivity and secretion altering supply of insulin to insulin-sensitive tissue and secretagogues to pancreatic islets. A decrease in local blood flow has been reported to arise insulin resistance. In non diabetic and type 2 diabetic subjects from Japan both the E298D (Glu298Asp) and the T-786C eNOS gene polymorphisms have been associated with insulin resistance. To our knowledge, no such date are available in Caucasian populations. Therefore we have examined the association of these polymorphisms with fasting and post-challenge glucose and insulin levels in 278 caucasian non diabetic men with essential hypertension.

Materials and Methods: A 75 g glucose tolerance test was performed and blood sampling were obtained at baseline and at 30′, 60′, 90′, 120′ and 180′ after glucose loading. Blood glucose and insulin (RIA method) were measured. Genotypes of both Glu298Asp and T-786C mutations were examined by the PCR-RFLP method.

Results: The percentages of the EE298, ED298 and DD298 genotypes were 45.0, 42.1 and 12.9%, respectively. The corrispective features for TT786, TC786 and CC786 were 21.4, 48.2 and 30.4%. Genotypes did not differ in age, BMI, waist-to-hip ratio, systolic and diastolic blood pressure and lipid profile. Fasting and post-challenge glucose and insulin levels did not differ by genotypes. No significant differences by genotypes were observed in homeostasis model assessment for insulin resistance (HOMA-IR: E298D: EE 3.0±1.9; ED 3.6±3.2; DD 2.8±1.2, p=0.10; T786C: TT 3.3±2.7; TC 3.3±2.7; CC 3.2±1.7, P=0.98) as well as in areas under the curve (AUC) for both glucose and insulin, or in insulinogenic index (DeltaI30/DeltaG30: E298D: EE 98±58; ED 135±132; DD 100±69, p=0.22; T786C: TT 112±83; TC 116±113; CC 120±99, P=0.91). No interaction between the two polymorphisms was observed: fasting and post-challenge glucose and insulin, AUC for glucose and insulin, HOMA-IR and the insulinogenic index did not differ by haplotypes.

Conclusion: These data suggest that in non diabetic hypertensive caucasian men the eNOS Glu298Asp and T-786C polymorphism did not affect insulin sensitivity or secretion as measured by oral glucose tolerance test.

0354

A systematic review and meta-analysis of the relationship between lipoprotein lipase gene Asn291Ser variant and dyslipidaemia

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Aims: Several case-control studies have examined the association between the Asn291Ser variant in the lipoprotein lipase gene and dyslipidemia, but most had limited power and results could therefore be conflicting. This systematic review attempts to summarize the association between the Asn291Ser variant in the lipoprotein lipase gene and dyslipidemia.
Methods: We systematically reviewed the literature by means of a meta-analysis. Meta-analysis were performed on RevMan4.2.8. To check for publication bias, a funnel plot was constructed. Three-dimensional structure of the human lipoprotein lipase was reconstructed based on known crystal structure of human protein lipase.
Results: Thirty-three family or case-control studies including 19246 white subjects were selected for the meta-analysis. Funnel plots showed no asymmetry, neither visually nor in terms of statistical significance($p>0.05$), indicating no publication bias. The summary standardised mean difference (SMD) of plasma triglyceride for carriers compared to non-carriers of the Asn291Ser variant was 3.23 (95% C.I. 2.49–3.97, $p<0.00001$). The summary SMD of plasma HDL-C for carriers compared to non-carriers of the Asn291Ser variant was - 3.42 (95% C.I. – 5.07–1.77, $p<0.0001$). The summary SMD about the association of the Asn291Ser variant with plasma triglyceride increased with the age of cases. The SMD raised from 0.92 (95% C.I. 0.20–1.64) for case subjects aged 35 to 45 years to 3.25 (95% C.I. 1.83–4.67) for case subjects that were 65 to 75 years old.
Conclusions: This meta-analysis indicates that the Asn291Ser variant in the LPL gene is a risk factor for dyslipidemia, characterized by hypertriglyceridemia and low HDL-C levels. And the Asn291Ser variant in the LPL gene predispose to more severe dyslipidemia with the increasing age.

0355

4G/5G polymorphism of plasminogen activator inhibitor (PAI-1) influences the relationship between C-reactive protein and PAI-1 in type 2 diabetic patients

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Background and Aims: C-reactive protein (CRP), in addition to being a proinflammatory biomarker, has been identified as a possible factor able to promote atherosclerosis. "In vitro" studies have demonstrated that CRP induces plasminogen activator inhibitor type 1 (PAI-1) expression, suggesting a hypofibrinolytic role for CRP. As CRP and PAI-1 levels increase in type 2 diabetic subjects, we decided to study the relationship between CRP and PAI-1, and the role of the 4G/5G polymorphism of the PAI-1 gene on this relationship in a diabetic population without complications.
Materials and Methods: Two hundred and ninety five type 2 diabetic patients (age 60.9±10.5 years) and 290 healthy controls (age 59.2±11.5 years) were enrolled and CRP, PAI-1 antigen and 4G/5G polymorphism were detected.
Results: A significant correlation between PAI-1 and CRP in diabetic subjects was found ($r=0.45$, $p<0.001$), whereas no relationship was evident in the control subjects between these inflammatory markers. Multiple regression analysis highlighted that CRP is the only one significant variable of PAI-1 levels in diabetic subjects (partial $r=0.31$, $p<0.01$). Stratifying by genotype, a positive correlation between PAI-1 and CRP in 4G/4G (partial $r=0.64$ $p<0.001$) and 4G/5G (partial $r=0.47$, $p<0.001$) subjects was found, whereas no correlation in 5G/5G was present. Multiple regression analysis confirmed the presence of this correlation in 4G/4G (partial $r=0.45$, $p<0.001$) and in 4G/5G (partial $r=0.34$, $p=0.007$) diabetic patients.
Conclusions: These findings demonstrate that CRP plays an important role in the complex mechanism in the regulation of PAI-1 levels in 4G diabetic carriers, supporting the hypothesis that CRP may have a direct role in diabetic atherothrombosis.

PS 11

Genetics of insulin resistance and cardiovascular disease

0356

Identification of functional protein kinase Cβ promoter polymorphisms related to insulin-resistance in non-obese humans

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Background and Aims: Insulin-resistance is a marker of impaired glucose metabolism and a risk factor for the

development of type 2 diabetes mellitus (T2DM). The alternative splicing of protein kinase Cβ (PKCβ) into subtypes β1 and β2 is affected by insulin and itself plays and important role in the regulation of insulin production in β-cells and the insulin depdendent glucose uptake in skeletal meuscle cells. We investigated five polymorphisms (SNPs) within the PKCβ gene (PRKCB1) regarding their influence on insulin-sensitivity in humans.

Materials and Methods: Promoter-assays were carried out by use of a promoter-fragment of PKCβ cloned into the pGL3-basic vector (Promega) containing either the frequent or rare allele of the polymorphism. We investigated 1068 caukasian individuals recruited in the region of Berlin and Potsdam, Germany. The cohort was categorized according to WHO-criteria into controls, patients with IFG, IGT and T2DM. The age was 52.2±14.3a, the BMI 28.8±6.3 kg/sqrm. The subjects were characterized by OGTT, anthropometric and biochemical parameters. The genotype of the SNPs was determined by TaqMan technology.

Results: We identified 2 polymorphisms in the promoter of PKCβ related to an decreased protein-expression of the enzyme and to insulin-resistance in humans. PKCβ promoter assays with w.t. and at position −546 mutated constructs showed an up to 4-fold lower activation of the mutated PKCβ promoter. Consequently, we found a significantly 3.4-fold decreased protein-expression of PKCβ in human subjects carrying the homozygously mutated variant at position −546. The −546 PKCβ promoter polymorphism was in strong linkage with another polymorphism at position −1504 (D́=0.958, p<0.00001) and both were associated significantly with increased fasting insulin concentrations (FI) and HOMA-IR in non-obese subjects (BMI <30) with impaired glucose metabolism (IGM). The fasting glucose levels were not altered. In obese subjects (BMI≥30) the FI levels and HOMA-IR were already increased and the polymorphisms had no additional effect.

Conclusion: The mutation of the PKCβ promoter at positions −546 and −1504 might lead to a reduced expression of the enzyme leading to insulin resistance, because of an impaired signal transduction in skeletal muscle- and β-cells altering glucose uptake and insulin production.

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0357

The common 3′UTR G>A variant of *CTLA4* associates with reduced fasting and post- OGTT insulinaemia in the general population: a study of 5,762 middle-aged whites

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Background and Aims: A widespread 3′UTR G>A polymorphism in the gene encoding the cytotoxic T lymphocyte antigen 4 (CTLA4), which acts as a potent negative regulator of T-cell responses, has been shown to associate with type 1 diabetes. This variant has been shown to influence the expression of the *CTLA4* gene. We hypothesised that the A-allele of the *CTLA4* variant rs3087243 modulates pancreatic β-cell function in the general population. Thus, the aim of the present study was to investigate the relationship between the *CTLA4* 3′UTR G>A polymorphism and fasting and post-OGTT insulinaemia in a sample of Danish whites.

Materials and Methods: The *CTLA4* 3′UTR G>A rs3087243 variant was genotyped in a population-based sample (Inter99) of 5,762 middle-aged subjects (2,865 men, 2,897 women, age 46±8 years (mean±SD), BMI 26.3±4.6 kg/m^2). All subjects underwent a standard 75 g oral glucose tolerance test (OGTT). Genotyping of the *CTLA4* variant was performed using chip-based matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry.

Results: The minor A-allele of the *CTLA4* 3′UTR G>A variant associates with decreased fasting and 2-hour post-OGTT serum insulin, and an increase in BMI (see table). In a case-control study the A-allele was less frequent among 2,548 subjects with BMI <25 kg/m^2 (40.1; 95%CI 38.7–41.4 (36% GG; 48% GA; 16% AA)) than among 952 participants having BMI ≥30 kg/m^2 (43.9; 95%CI 41.6–46.1 (32% GG; 49% GA; 20% AA), *P*=0.005). Thus, the variant associates with obesity with an odds ratio of 1.2 (95%CI 1.1–1.3).

	G/G	G/A	A/A	*P*
N (men/women)	1,985 (1,007/978)	2,772 (1,356/1,416)	1,005 (502/503)	
Age (years)	46±8	46±8	46±8	
BMI (kg/m^2)	26.0±4.4	26.3±4.5	26.5±4.8	0.003
Plasma glucose				
Fasting (mmol/l)	5.5±0.8	5.5±0.7	5.6±1.0	0.2
120-min post-OGTT (mmol/l)	6.3±2.2	6.2±2.1	6.3±2.1	0.06
Serum insulin				
Fasting (pmol/l)	43±29	41±27	42±27	$5{\cdot}10^{-5}$
120-min post-OGTT (pmol/l)	226±235	211±203	217±203	0.002

Data are mean±SD. A general linear model was applied. *P* values were adjusted for sex, age, and BMI (where appropriate) and were calculated assuming a dominant model.

Conclusion: The widespread *CTLA4* 3′UTR G>A polymorphism associates with significantly reduced fasting and 2-hour post-OGTT serum insulin levels in middle-aged whites, suggesting that variation in a type 1 diabetes susceptibility gene may modulate insulinaemia in the general population of middle-aged people. Moreover, our findings suggest that the *CTLA4* variant may associate with a modest increase in BMI. Further studies are needed to confirm this finding and to explore the mechanism by which variation in the *CTLA4* locus may contribute to BMI regulation.

0358

The Q130E polymorphism in the Caveolin-2 gene is associated with biological features of hepatic steatosis and postprandial insulin levels

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Background and Aims: Type 2 diabetes in humans is associated with increased de novo lipogenesis, decreased fatty acid oxidation and hepatic steatosis. Caveolae are small membrane invaginations on the surface of cells that participate in membrane trafficking, sorting, transport and signal transduction. Caveolin-2 is a principal component of caveolae membranes in many cell types, with particular high levels in adipocytes, smooth muscle and the liver. The specific overexpression of Cav-2β results in the constitutive localization of Cav-2β to the surface of lipid droplets, suggesting a role for this Cav-2 isoform in lipogenesis. As caveolin-2 could be an enhancer for hepatic lipogenesis, variations in this gene could lead to abnormal hepatic lipid accumulation and insulin resistance. We investigated the association between different caveolinpolymorphisms and hepatic and metabolic parameters in Metabolic Intervention Cohort Kiel (MICK) to elucidate this question.
Materials and Methods: A total of 716 male subjects from a postprandially characterised cohort (MICK) were genotyped for the Caveolin-2 polymorphism (rs8940). Subjects underwent an oral glucose tolerance test and a standardised mixed meal. Glucose, insulin, triglycerides were determined after the mixed meal, glucose and insulin after the glucose load. Increased liver enzymes were used as surrogates of liver steatosis.
Results: In comparison to homozygote subjects of the wild allele, carriers of the less common allele showed significantly lower levels of alanine aminotransferase and aspartat aminotransferase (p=0,006, p=0,019 rsp.), lower postprandial insulin levels after a standardised mixed meal (OMTT) (p=0,018) and higher postprandial insulin sensitivity (HOMA AUC, p=0,04). BMI was not significantly different in both groups and adjustment for this confounder did not attain the results.
Conclusion: These results suggest that the rare allele of a Caveolin-2 gene polymorphism may be protective against insulin resistance and hepatic steatosis.
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0359

Single nucleotide polymorphisms in the procolipase gene are associated with insulin sensitivity and insulin secretion in humans

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Background and Aims: Procolipase (PCLPS) is secreted from the pancreas during fat digestion into the gastrointestinal tract. After removal of the signal peptide, PCLPS is proteolytically cleaved into colipase (CLPS) and the pentapeptide enterostatin (ES). CLPS is an indispensable activating cofactor for pancreatic lipase and therefore permits lipid digestion in the gut. By contrast, ES acts as a signaling molecule that inhibits insulin secretion and confers satiety signals to the brain, thereby restricting further food intake in animal models. As both high fat diet and obesity contribute to insulin resistance, we were interested whether single nucleotide polymorphisms (SNPs) of the human *PCLPS* gene are associated with insulin sensitivity, insulin secretion and serum lipids in humans
Materials and Methods: A total of 1500 base pairs of the promoter, all 3 exons, exon–intron boundaries and the 3′UTR of *PCLPS* were analyzed by bidirectional sequencing in 50 non-diabetic subjects. Among the identified SNPs, those with an allelic frequency>5% were genotyped in 498 non-diabetic subjects.
Results: Two SNPs, −3 *A/G* (rs3748050 in the Kozak sequence) and+122 *A/G* (rs3748051 in intron 1) were in tight linkage disequilibrium. Both the G allele of the −3 *A/G* SNP (allele frequency 0.24) and the G allele of the+122 *A/G* SNP (allele frequency 0.23) were associated with higher insulin sensitivity estimated from the oral glucose tolerance test [OGTT as estimated by Matsuda and DeFronzo, (e.g. −3 *A/G*, AA 14.68±0.69, AG 15.86±0.81 and GG 18.68±2.17 arb. units, mean±SE, p=0.02)], independent of age, gender and body fat. Moreover, they were

associated with lower insulin secretion as determined by C-peptide levels at minute 30 of the OGTT (e.g. −3 *A/G*, AA 1837±47, AG 1789±53 and GG 1587±98 pM, p=0.02), after additional adjustment for insulin sensitivity. Further, haplotype analyses confirmed the relevance of both SNPs for these phenotypes. No significant associations with fasting total cholesterol (c), HDL-c, LDL-c, triglycerides and free fatty acids were found (all p>0.11).

Conclusion: These data support that *PCLPS*, encoding both an enzyme (CLPS) and concomitantly a hormone (ES), has different functions on insulin sensitivity and insulin secretion. While the G alleles of these SNPs may have beneficial effects on insulin sensitivity, particularly their inhibitory effects on insulin secretion may be important in subjects at high risk for the development of type 2 diabetes.

0360

Heritability of serum resistin and its genetic correlation with insulin resistance-related features in non-diabetic Caucasians

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Background and Aims: Resistin, a cysteine-rich peptide, is believed to play a role in several metabolic pathways and inflammatory responses. Serum levels of resistin are believed to modulate insulin resistance in humans.
The aim of this study was to investigate whether serum resistin levels are genetically controlled and whether this control is shared with other insulin resistance traits.

Materials and Methods: The study cohort included 264 non diabetic probands, Caucasian from Italy, and their 473 adult family members. Phenotypic characterization included anthropometric variables, blood pressure, fasting glucose and insulin, lipid profile and resistin levels. Genotypes were determined at position g.-420C>G (rs1862513), IVS2+181G>A (rs3745367), and $GAT_{(n)}$ polymorphisms of the resistin *(RETN)* gene.

Results: In the 264 unrelated probands, resistin levels were significantly (p<0.01) correlated with adiposity, blood pressure, C-reactive protein, and the metabolic syndrome score. In a variance component analysis of the 264 probands and their 473 relatives, about 70% of the observed variation of serum resistin levels was heritable (p<0.0001). A small, but significant (p=0.004) proportion of this heritability was explained by the G>A variation at position IVS2+181 of the *RETN* gene. Significant genetic correlations (p<0.05) were observed between resistin and BMI (ρg=0.30), waist circumference (ρg=0.32), the insulin resistance index $HOMA_{IR}$ (ρg=0.28) and the metabolic syndrome score (ρg=0.35).

Conclusion: These data indicate that serum resistin is highly heritable and has some common genetic background with traits related to insulin resistance, reinforcing the hypothesis that this adipokine may play a pathogenic role in insulin resistance-related abnormalities, including type 2 diabetes and cardiovascular disease.

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0361

Genetic association of Pro197Leu allele at *GPX-1* gene to coronary artery calcification suggests importance of shifted redox balance for development of atherosclerosis in diabetes

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Background and Aims: Recent studies suggest that increase in reactive oxygen species (ROS), other than the classical Framingham factors, is a possible explanation for why diabetes mellitus promotes arterial sclerosis, specifically coronary artery disease. However, its mechanisms that shift the redox balance and determine individuals’ susceptibility to coronary artery sclerosis in diabetes remain unknown. To know it, we analyzed SNPs of genes for enzymes that could scavenge and reduced ROS, as well as elements that could increase ROS and promote susceptibility to atherosclerosis in diabetes.

Materials and Methods: Eighty-six patients with type 2 diabetes (42–81 years olds, M/F=60/26) were enrolled in this study, and were evaluated on coronary artery calcium score (Agaston CACS) by 16-detector Multi-Slice CT (MSCT). The patients were genotyped for ROS-scavenging enzymes; *Glutathione peroxidase1 (GPx-1), Catalase, Mn-SOD and Cu/Zn-SOD*. The patients were also evaluated for ROS-promoting elements including SNPs of *NADPH*, age, blood pressure, BMI, glycemic control, lipid metabolism and duration of diabetes. Genotypes were determined by PCR-based methods. All clinical values are expressed by mean±SD. Statistical analyses were performed by t-test, χ^2-test and multiple regression analysis using Dr.SPSS according to patients’ groups divided based on CACS, or

genotype. The genetic association test for deviation from the Hardy–Weinberg equilibrium was performed by the DeFinetti program.

Results: As shown in Table, only the CACS score of *GPx-1* gene was significantly disparate; CACS for Pro/Leu heterozygotes was higher than that of Pro/Pro homozygotes ($p<0.002$). Further analysis for *GPx-1* gene showed that genotype frequency of Pro/Leu of patients' group with CACS over 1000 was significantly higher than that of CACS under 999 (45.5% vs. 17.3%) when analyzed by χ^2 test. Moreover the test for deviation from Hardy–Weinberg's equilibrium also confirmed the association (OR=3.97, CI=1.05-15.01; p=0.032). Finally, multiple regression analysis for CACS to patients' parameters including SNPs revealed presence of significant correlation with *GPx-1* genotype and age to CACS.

Gene	Genotype	frequencies	CACS	p value
GPx-1	P/P	68/86 (79.1%)	234±396	0.002 *
(Pro197Leu)	P/L	18/86 (20.9%)	824±1340	
Catalase	T/T	20/83 (24.1%)	323±615	0.755
(SNP-89)	A/T & A/A	63/83 (75.9%)	384±787	
Mn-SOD	V/V	46/83 (55.4%)	317±688	0.483
(Val16Ala)	V/A & A/A	37/83 (44.6%)	433±817	
Cu/Zn-SOD	C/C	30/82 (36.6%)	388±825	0.895
(intron 3)	C/A & A/A	52/82 (63.4%)	365±711	
NADPH	C/C	72/83 (86.7%)	324±765	0.16
(+242)	T/T & T/C	11/83 (13.3%)	666±544	

Conclusion: Presence of Pro197Leu variant allele of *GPx-1* could determine genetic susceptibility to coronary artery sclerosis in type 2 diabetes by low scavenging ability of ROS.

0362

Prohormone convertase gene variants and proinsulin concentrations

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Background and Aims: Recently, it was shown that proinsulin is a significant predictor of Type 2 diabetes (T2DM), independent of insulin resistance and insulin secretion. Proinsulin is converted to insulin by concerted action of two sequence-specific proteases termed prohormone convertase 1 (PC1) and 2 (PC2). PC1 initiates the sequential processing of proinsulin to insulin by cleaving the proinsulin molecule where the B-chain and C-peptide join. PC2 and Caboxypeptidase E (CPE) catalyze further reactions. Expressions of PC1 and insulin are co-ordinately regulated by glucose. Thus, PC1 plays a key role in regulating insulin biosynthesis.

To examine if SNPs in the PC1, PC2 and CPE genes are associated with proinsulin and insulin concentrations.

Materials and Methods: We investigated genotypes, SNPs, selected from public databases, using multiplex PCR at the WCN-Platform North, Uppsala in relation to proinsulin and insulin determinations using data from the ULSAM cohort.(www.pubcare.uu.se/ULSAM). Also haplotypes (HTs), estimated using the software PHASE 2.1, were investigated. Permutations were used to control for inflation of the alfa-error.

Results: A SNP in an exon of the PC1 gene showed 50% higher proinsulin concentrations in homozygotes as compared to wildtype (p for trend <0.001) and in a HT (n=12/545, p for trend <0.001). A SNP in an exon of the PC2 gene showed 25% higher proinsulin concentrations in homozygotes as compared to wildtype (p for trend 0.021) and 150% higher proinsulin concentrations in a HT (n=14/533, p for trend 0.002). This haplotype was borderline associated with an increased risk for T2DM (OR=3.1, p=0.057). For CPE, results were non-significant.

Conclusion: These data suggest that genetic variation in the PC1 and PC2 genes is associated with proinsulin concentrations.

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0363

The ACE DD genotype is associated with a high risk for fatal myocardial infarction in people with type 2 diabetes and high cardiovascular risk

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Background and Aims: The insertion/deletion (I/D) polymorphism of the angiotensin converting enzyme (ACE) gene is a validated risk factor for diabetic nephropathy. The prognostic value of the D allele for acute myocardial infarction (AMI) was only established on case-control studies, seldom studying type 2 diabetic patients. The aim of our prospective study was to evaluate its prognostic value in a large population of type 2 diabetic patients.

Materials and Methods: The DIABHYCAR trial is a randomised controlled trial comparing ramipril 1.25 mg/day with placebo, for type 2 diabetic patients with high cardiovascular risk (micro- or macro-albuminuria, age>50 years). The main outcome was the occurrence of a cardiovascular event including fatal or non-fatal AMI. Here, the history of myocardial infarction at baseline, the occurrence of an AMI and the post-AMI mortality were examined in 3139 French subjects, taking part in this study, according to the ACE I/D polymorphism.
Results: At baseline, history of AMI was less frequently encountered in DD genotype subjects, compared with other participants : DD, 47/1119 (4.2%), ID, 85/1468 (6.1%) and II, 39/552 (7.1%) (p=0.0384). This lower risk persisted after adjustment on sex, age, blood pressure, cholesterol, smoking, HbA1c (DD vs ID or II: adjusted odds ratio 0.64, 95% CI: 0.44–0.94; p=0.02). During the study follow-up (4 years), 95 AMI occurred (3%) and this incidence was not associated with the I/D polymorphism. The post AMI mortality was significantly different according to the polymorphism : 7/28 DD genotype patients died (25%) vs 3/49 ID genotype subjects (6.1%) and 1/18 II genotype subjects (5.6%) (p=0.03). This increased risk of DD subjects persisted after adjustment on the above cited risk factors (DD vs ID or II : adjusted hazard ratio 11.2, 95% CI 2.1–58.2; p=0.04).
Conclusion: The DD genotype of the ACE I/D is not a risk factor for AMI but is associated with an increased risk of post- AMI mortality, in type 2 diabetic subjects, which may lead to a survival bias that might account for spurious results in cross-sectional analyses.

0364

Effect of ENPP1 (PC-1) K121Q polymorphism on metabolic syndrome

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Background and Aims: Ectonucleotide pyrophosphatase/ phosphodiesterase 1 (ENPP1), also named plasma cell membrane glycoprotein (PC-1), is a promising candidate gene for type 2 diabetes because it inhibits autophosphorylation of the insulin receptor. The ENPP1 K121Q polymorphism has been associated with insulin resistance in some but not in all studies.
In this report we analyze in an adult (35–74 years) Caucasoid population (n=794) from a cross-sectional population-based epidemiological survey in Central Spain (Segovia, A.C Castille), including type 2 diabetics (n=73, 9.2%), whether the K121Q polymorphism of the ENPP1 gene is associated with increased risk for the development of obesity, insulin resistance and other features of the metabolic syndrome as defined by the 2005 IDF criteria.
Materials and Methods: Anthropometric parameters: BMI, waist circumference. OGTT, fasting and 2 hrs glucose; lipid profile, serum insulin, proinsulin, leptin, adiponectin levels. Insulin resistance was assessed by HOMA-IR. ENPP1 K121Q genotypes determined by PCR-RFLP.
Results: Glucose Tolerance: Normoglycemia 564 (71%), Impaired Fasting Glucose+Impaired Glucose Tolerance 157 (19.8%), Type 2 Diabetes 73 (9.2%). ENPP1 genotype distribution: K121K 73.2%, K121Q 24.8%, Q121Q: 2.0%. Overall prevalence of metabolic syndrome was 25.1%; 24.3% in males and 25.7% in females. The Q allele was associated with higher BMI (31.3±3.0 vs. 30.0±3.6, P=0.027) and waist circumference (101±7.2 vs. 98±9.3, P=0.043) among subjects with metabolic syndrome compared with K121K genotype. Those findings were also significant even after the adjustment for sex, age, and degree of glucose tolerance (β=1.347, P=0.017 and β=2.824, P=0.046; for BMI and waist circumference respectively).
Conclusion: The K121Q PC-1 polymorphism did not appear to be associated with insulin resistance in Caucasoid Spaniards although in subjects with metabolic syndrome the Q allele was associated with obesity related parameters such as a higher BMI and waist circumference.

0365

The role of Leu72Met and -604 C>T polymorphisms of the ghrelin gene on insulin resistance in Italian population

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Background and Aim: Ghrelin (GHRL) is a circulating 28-amino acid gastrointestinal and neuropeptide that is widely expressed in many tissues and primarily produced by the stomach. Circulating ghrelin levels are correlated with obesity and mutations of the gene could influence the function of this hormone and might alter the energy balance. Previous studies, suggested that polymorphisms in the coding region of the preproghrelin were involved in the aetiology of obesity and might modulate glucose induced insulin secretion.
The aim of our study was to evaluate the association of the three GHRL polymorphisms: -604 C>T, Leu72Met and

Gln90Leu, with obesity and measures of insulin sensitivity in 1420 individuals.

Materials and Methods: The three SNPs were genotyped using ABI PRISM 7900 HT Sequence Detection System. We assumed specific co-dominant genetic model and we used multiple linear regression analysis for quantitative traits. Human plasma ghrelin was measured with a commercial radioimmunoassay (Phoenix) in 300 overweight/obese subjects. The plasma samples were collected under fasting conditions.

Results: We observed a significant decreasing trend from Leu/Leu homozygous, to Leu/Met heterozygous and to Met/Met homozygous for triglycerides, fasting insulin levels and HOMA-IR index (p=0.02, p=0.01 and 0.001, respectively). Consistently with these findings a significant increasing trend was observed in ghrelin levels for three category genotypes (29.2 pg/ml, 32 pg/ml and 36.64 pg/ml, p=0.007).

The -604 T>C SNP was analyzed in the whole cohort determining a trend of decrease from CC to TC and to TT for insulin levels and HOMA-IR index, however the data was not statistically significant (p=0.08 for both). Nevertheless the same analysis was performed in subjects homozygous for Leu72 and we noted a significant decreasing trend in insulin levels and HOMA-IR index (p=0.001 for both). No difference was observed in ghrelin levels between the groups. There were no significant associations between Gln90Leu polymorphism and investigated variables.

Conclusions: Our observations suggest that -604 C>T and Leu72Met ghrelin gene polymorphisms modulate insulin sensitivity. Interestingly we found that the -604 T allele influences insulin sensitivity only in subjects not carrying the Met72 variant.

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PS 12

Cardiovascular risk factors

0366

Insulin resistance assessed by homeostasis model assessment predicts incident symptomatic cardiovascular disease in Caucasians subjects from the general population. The Bruneck Study

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Background and Aims: Insulin resistance is related to several classic and non-traditional risk factors. Aim of the present study was to evaluate whether insulin resistance can independently predict incident cardiovascular disease (CVD) events in the general population.

Materials and Methods: A sample representative of the general population of Bruneck, Italy (n=919; age 40–79 years) had an estimate of insulin resistance with the Homeostasis Model Assessment (HOMA-IR) and the measure of several candidate risk factors (smoking, BMI, blood pressure, HbA1c, lipids, apoproteins, urate, adiponectin, inflammatory markers, endothelial adhesion molecules). Subjects were followed-up for 15 years. CVD was a composite end-point including fatal and non-fatal myocardial infarction and stroke, transitory ischemic attack, and any revascularization procedure. Subjects without insulin measurement (n=31) or pre-existing CVD (n=49) were excluded from analyses.

Results: Insulin resistance was correlated to most classic and non-traditional risk factors also after adjusting for sex, age, BMI and smoking. During the 15 years of follow-up, 118 subjects experienced one or more symptomatic CVD events. Subjects of top quartile of HOMA-IR (i.e., insulin resistant subjects) had an increased risk of incident symptomatic CVD as compared to subjects belonging to other three quartiles (i.e., non-insulin-resistant subjects). This was found in the model including only demographic variables (sex and age), in the model including also physical activity and classic risk factors (smoking, HbA1c, LDL-cholesterol and hypertension), and in the model including also BMI, HDL-cholesterol and triglycerides. Moreover, when the model included also non-traditional risk factors (fibrinogen, oxidized LDL, hsCRP, E-selectin, adiponectin), the association between HOMA-IR and CVD remained significant and virtually unchanged (HR 2.4, 1.5–3.8, p<0.001). Results were unchanged when subjects with IFG, IGT or diabetes were excluded. Results were similar in men and women.

Conclusion: Insulin resistance, as assessed by a simple method based upon the measurement of fasting plasma glucose and serum insulin, seems to be able to predict subsequent symptomatic CVD in the general population also independently of several classic and non-traditional risk factors related to this metabolic phenomenon.

0367

Screen detected subjects with type 2 diabetes and impaired glucose tolerance have more adverse cardiovascular risk than subjects with impaired fasting glucose

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Background and Aims: Not only type 2 diabetic patients but also subjects with impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) are at increased risk of cardiovascular diseases (CVD). However, there is debate regarding the strength of the association between IFG and CVD risk. In addition, differences in cardiovascular risk factors between different categories of impaired glucose regulation may have implications for screening strategies. Our aim was to assess and compare the cardiovascular risk profiles of subjects with type 2 diabetes, IGT and IFG identified in a screening programme.
Materials and Methods: From 2002 to 2003, 29,251 nondiabetic patients, aged 50–70 years, from 41 primary care practices, were invited to participate in a population-based diabetes screening programme in the Netherlands. The screening was part of the ADDITION study (*Anglo–Danish–Dutch Study in general practice of intensive treatment and complication prevention in type 2 diabetic patients identified by screening)*. Clinical characteristics were assessed of 285 detected diabetic patients, 175 subjects with IGT, 218 with IFG, and 72 subjects with normal glucose tolerance (NGT).
Results: Body mass index (BMI) and systolic (SBP) and diastolic (DBP) blood pressure were significantly different between subjects with NGT and IFG on the one hand and subjects with IGT and diabetes on the other hand (*NGT*: BMI (kg/m^2) 26.7±SD 3.7, SBP (mmHg) 150±25, DBP (mmHg) 84±10; *IFG*: BMI 27.3±4.4, SBP 150±25, DBP 84±12; *IGT*: BMI 29.5±5.7, SBP 161±24, DBP 89±12; *type 2 diabetes*: BMI 30.7±5.6, SBP 162±23, DBP 90±11). Poor glycaemic control was associated with elevated BMI, blood pressure and lipids. In subgroup analyses the age and gender adjusted association between glycaemic control and SBP, HDL-cholesterol, LDL-cholesterol and triglycerides were still significant in subjects with BMI>27 kg/m^2, but not any longer in those with BMI <27 kg/m^2. Such effect modifying by BMI was not found regarding DBP. In subjects with BMI>27 kg/m^2 HbA1c, blood pressure and lipids were more adverse than in those with BMI <27 kg/m^2.
Conclusion: Both screen detected type 2 diabetic patients and subjects with IGT had an unfavourable cardiovascular risk profile. Blood pressure and weight in subjects with IFG were significantly lower than in subjects with type 2 diabetes and IGT suggesting IFG is a condition with less risk to develop cardiovascular diseases. The increased CVD risk of hyperglycaemia is notably present in obese persons. Our data indicate that it might be more appropriate to screen for overweight rather than for hyperglycaemia in order to detect people with increased cardiovascular risk.

0368

Two-hour post-load glucose: an independent predictor of 5-year all-cause mortality – the AusDiab study

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Background and Aims: Hyperglycaemia, in particular elevated post-load glucose, has been identified as an important predictor of mortality. Some studies have indicated that even smaller elevations below the diabetic threshold are independently associated with mortality. However, few data exist from nationally representative cohorts which have incorporated an oral glucose tolerance test (OGTT). Therefore, the aim of this analysis was to investigate, in people without previously diagnosed diabetes, whether post-load plasma glucose is an independent predictor of all-cause mortality.
Materials and Methods: The Australian Diabetes, Obesity and Lifestyle Study (AusDiab) is a nationally representative population-based survey of adults aged ≥25 years. Baseline (1999–2000) measurements for standardised HbA_{1c}, fasting and post-load plasma glucose (from a 75 g OGTT) were available for 9935 people without previously diagnosed diabetes. In 2005, vital status was ascertained from the National Death Index.
Results: Over a median of 5.1 years, 247 deaths were identified. After adjusting for age and sex, the hazard ratios (HRs) across increasing quintiles for post-load plasma glucose were 1.5 (95%CI 0.9–2.7) for 4.7–5.4 mmol/l, 1.7 (1.0–3.0) for 5.5–6.2 mmol/l, 1.4 (0.8–2.4) for 6.3–7.4 mmol/l, and 1.9 (1.1–3.1) for 7.5–28.7 mmol/l (P=0.04 for trend). After further adjustment for other known risk factors, the hazard ratios (HRs) for increasing quintiles were 1.5(0.9–2.6), 1.8(1.0–3.0), 1.5(0.9–2.6), and 1.9(1.2–3.2) (P=0.025 for trend). After fasting blood glucose and/or HbA_{1c} were included in the multivariate model, the HRs for increasing quintiles of post-load glucose were unchanged. However, while the overall trend for post-load glucose remained significant with the inclusion of fasting blood glucose (P=0.038 for trend), the trend showed only borderline significance when HbA_{1c} was included (P=0.06).
Conclusion: Over a 5-year period, post-load plasma glucose, even at levels below the diabetic threshold, is an independent predictor of all-cause mortality in people without previously diagnosed diabetes. HbA_{1c} and/or fasting plasma glucose did not alter the mortality risk

associated with post-load plasma glucose. The results of this study provide further support for the utility of post-load hyperglycaemia in predicting total mortality in people without diabetes.

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0369

Proinsulin predicts development of cardiovascular risk factors in a 7-years population-based follow-up study

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Background and Aims: Proinsulin is released in increased amounts in persons who are at cardiovascular risk or who develop type 2 diabetes. In fact, it has been speculated that proinsulin may have a pathogenetic role in the development of cardiovascular disease. Most studies have been performed in persons at increased risk of disease or in patients with established disease, and preferentially in men. We therefore posed the question whether proinsulin predicts development of cardiovascular risk in low-risk subjects, regardless of gender.

Materials and Methods: In a population-based study of 1.946 non-diabetic men (age 59.0 (9.2), average (SD)) and 1.926 non-diabetic women (age 59.6 (9.8)) we measured albumin:creatinine ratio (ACR) in three morning urine samples, as well as HbA1c, lipids, blood pressure and body weight, height and waist circumference. The measurements were performed both in 1994–5 and in 2001–2. Development of cardiovascular risk factors in relation to quartiles of proinsulin (as measured in 1994–5) was analyzed in a multiple logistic regression analysis. Cardiovascular risk factors were defined as: HbA1c>6.0%, dyslipidemia (triglycerides>1.7 mmol/l and/or HDL-cholesterol <0.9 mmol/l (men) or <1.0 mmol/l (women), ACR>0.7 mg/mmol, and hypertension (blood pressure ≥140/90 mmHg or pharmacological antihypertensive treatment). In each analysis the risk factor was not present in 1994–5.

Results: Over this 7-years period baseline proinsulin (highest vs. lowest quartile) predicted increase in HbA1c>6.0% both in men and women (OR men 2.95, 95% CI: 1.44, 6,06; OR women 2.42, 95% CI: 1.16, 5.05) and dyslipidemia in both genders (OR men 1.65, 95% CI: 1.04, 2,26; OR women 1.88, 95% CI: 1.23, 2.90). Proinsulin predicted development of ACR>0.7 in men only (OR 2.08, 95% CI: 1.24, 3.50). There was no relationship between proinsulin and development of hypertension in neither men nor women. The association between proinsulin and development of cardiovascular risk factors was no longer significant when proinsulin was corrected for by insulin (proinsulin:insulin ratio). Insulin:glucose ratio, a surrogate parameter for insulin resistance, gave similar results as proinsulin alone (data not shown).

Conclusions: Proinsulin predicts the development of cardiovascular risk factors in both genders, with the exception of microalbuminuria, which is predicted only in men. Since essentially the same results were found with the insulin:glucose ratio, and the associations were no longer present when the proinsulin:insulin ratio was calculated, we conclude that the relationship between proinsulin and cardiovascular risk factors is related to insulin resistance, and probably not by specific effects related to proinsulin per se.

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0370

The cardiovascular risk in patients with type 1 diabetes mellitus and metabolic syndrome

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Background and Aims: The metabolic syndrome (MS) is an important risk factor for cardiovascular disease and premature mortality in patients with type 2 diabetes mellitus (DM) and nondiabetic persons. MS is a condition characterized by a clustering of clinical and biochemical proatherogenic abnormalities.The aims of our study were to estimate the prevalence of MS in patients with type 1 DM and to compare the cardiovascular risk present in these individuals with that of type 1 diabetic patients without MS. In addition, we assessed whether the presence of MS in type 1 DM is associated with impairment of renal function and poor glycemic control.

Materials and Methods: The study enrolled 724 patients with type 1 DM, 382 men (52.8%) and 342 women (47.2%), with the mean age of 42.3±7.8 years and the mean duration of DM 23.4±7.2 years. The MS was defined according to the new IDF criteria. The absolute 10 years risk of developing

coronary heart disease (CHD-Risk) was assessed using the Coronary Chart Euro '98. Glomerular filtration rate (GFR) was estimated with the Cockcroft-Gault equation. Glycemic control was classified as good (HbA1c <6.5%), intermediate (HbA1c=6.5–9%), or poor (HbA1c>9%).

Results: The MS was present in 212 patients (29.28%) with type 1 DM. The prevalence of MS was higher in women (32.75%) than in men (26.18%). The prevalence of MS increased with worsening of renal function: from 20.65%, in patients with GFR>60 ml/min/1.73 m^2, to 38.89%, in those with GFR=30–60 ml/min/1.73 m^2, and to 62.79%, in persons with GFR <30 ml/min/1.73 m^2. The prevalence of MS was 17.52% in patients with good glycemic control, 29.7% in those with intermediate and 46.03% in subjects with poor glycemic control. CHD-risk was 12.1% in patients with MS vs. 8.3% in those without MS.

Metabolic characteristics of type 1 diabetic patients with and without MS

	MS	Without MS	p
Number (M/F)	100/112	282/230	
HbA1c (%)	8.4±2.5	7.4±1.9	<0.001
Systolic blood presure (mmHg)	142.3±24.2	132.5±19.1	<0.001
Diastolic blood presure (mmHg)	92.4±10.7	81.3±9.3	<0.001
Total cholesterol (mg/dL)	231.2±42.3	203.4±38.2	<0.001
LDLc (mg/dL)	121.2±26.1	113.1±24.3	<0.001
HDLc (mg/dL)	33.6±8.7	46.7±9.9	<0.001
Triglycerides (mg/dL)	274.8±56.4	168.8±42.3	<0.001

Data are mean±standard deviation. P was calculated with unpaired Student's t test.

Conclusion: MS is more prevalent in individuals with type 1 DM than in general population and increases the cardiovascular risk of these patients. The prevalence of MS was higher in type 1 diabetic patients with renal function impairment and with poor glycemic control.

0371

Does screening for diabetes identify a group of diabetics with high risk of coronary heart disease and is the risk modifiable? The ADDITION study

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Background and Aim: A key factor in assessing the balance between the costs and benefits of implementing policies for early detection of Type 2 diabetes is the magnitude of cardiovascular risk reduction following early detection and intensive therapy. The ADDITION study includes 3000 people with screen detected type 2 diabetes in the Netherlands, the United Kingdom and Denmark in order to address this issue. The study is a pragmatic randomised controlled trial of the impact of intensified multi-factorial treatment on 5 year cardiovascular event rate compared to standard care in people with screen-detected diabetes. Here we describe the baseline coronary heart disease (CHD) risk of the first 2000 enrolled individuals and analyse the extent to which that risk is potentially modifiable.

Material and Methods: Stepwise screening strategies varied between the centres but included risk questionnaires, routine general practice data, random blood glucose, HbA1c and fasting blood glucose. All individuals with Type 2 diabetes were diagnosed using the 1999 World Health Organization (WHO) criteria.

Results: In the period April 2001 to March 2005, 299 general practices were recruited and 58,736 people entered the first step of the screening process in the three countries combined. Demographic, clinical and biochemical characteristics of the first 2000 people with Type 2 diabetes included in the trial are shown in Table. The average 10-year CHD risk (UKPDS engine) for the included women and men was respectively 13% (SD=7.5) and 24% (11.7). The 25% and 75% quartiles in women and men were 7.6–15.9% and 15.4–38.6% respectively. Ten percent of the women and men had a risk above 22% and 39% respectively. Of people with blood pressure>140/90, 57% were not in antihypertensive treatment. Correspondingly 91% of those with total cholesterol>5 mmol/l were not in lipid lowering treatment.

Conclusion: The population identified by screening in the ADDITION trial has a CHD risk profile similar to populations with traditionally identified diabetes[1]. There seems to be a realistic possibility to reduce this risk through lifestyle intervention and medical therapy especially as a

major proportion of people at risk is not on relevant pharmacological treatment.

[1]Song SH and Brown PM, Diabetic Medicine, 21, 238–245

Baseline characteristics. Standard deviations in brackets.

	Men	Women
	N=1153	*N=847*
Age at diagnosis, *N=2000*	59.4	60.5
% White, *N=1938*	97.2	96.4
HbA1c (%), *N=1885*	7.3 (1.7)	7.0 (1.5)
Systolic BP, mmHg, *N=1955*	154 (23)	153 (23)
Diastolic BP, *mm*Hg, *N=1955*	89 (12)	86 (12)
BMI (kg/m^2), *N=1930*	31.2 (5.2)	32.3 (6.3)
Waist, cm, *N=1932*	110 (12)	103 (14)
Total Cholesterol, mmol/l, *N=1941*	5.5 (1.1)	5.8 (1.1)
Triglyceride, mmol/l, *N=1937*	2.1 (1.7)	1.9 (1.1)
% with microalbuminuria (UAC ratio>3.4), *N=1799*	18.0	13.7

0372

The metabolic syndrome as predictor of coronary heart disease, all-cause mortality and insulin resistance – the influence of choice of criteria

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Background and Aims: Controversy exists regarding the most appropriate definition of the metabolic syndrome (MS). In addition to the recent IDF criteria, earlier definitions from the WHO and NCEP ATP III have been widely used in their complete form or truncated, due to lack of full criteria, in epidemiological studies. In a population-based investigation of elderly males, all three definitions could be applied, since data permitting the full definitions were collected at age 70, including measurements of insulin sensitivity with the euglycamic insulin clamp. We have compared the three definitions as predictors of coronary heart disease, all cause mortality, association with insulin sensitivity and consistency in defining the MS.
Materials and Methods: The subjects were participating in Uppsala Longitudinal Study of Adult Men. The baseline investigation was carried from August 1991 to May 1995 in 1221 men with a follow-up of up to 10 years using Swedish national registry data for CHD and all-cause mortality. The baseline investigation included anthropometry, OGTT, blood pressure, smoking, serum lipids, a euglycaemic insulin clamp and urinary albumin excretion. The outcome measure was defined as death or first time hospitalised for CHD. CHD was defined according to ICD 9 codes 410 to 414. Associations were analyzed using Cox's proportional hazards regression, presented as hazard ratios (HRs) with 95% confidence intervals (CIs) for a one SD increase in the predictor variable.
Results: In analyses adjusted for LDL-cholesterol and smoking, the the HR for CHD with MS defined according to the WHO, NCEP and IDF criteria at age 70, was 2.29 (95% CI;1.56–3.38;p<0.0001), 2.06 (1.37–3.10; p<0.0005) and 1,42 (0.95–2.13; p=0.088) and for all-cause mortality 1.67 (1.28–2.17), 1.64(1.23–2.19) and 1.56 (1.17–2.09), respectively. The insulin sensitivity index (M/I) was 3.35 (SD;1,74), 3.21 (1.75), 3.44(1.78) mg*min^{-1}*kg^{-1}/(100 mU/l, respectively. The mean M/I in men not defined as having the metabolic syndrome ranged between 5.64–6.20 mg*min^{-1}*kg^{-1}/(100 mU/l.
Altogether 536 (51.7%) men were defined as having the metabolic syndrome with either of the three definitions. Of those only 174 (32.5%) were defined by all three.
Conclusion: In elderly males the metabolic syndrome, as defined by WHO, NCEP III ATP III and IDF criteria was associated with increased all-cause mortality. For CHD incidence WHO, NCEP III, but not IDF criteria, were associated with approximately 2 times higher incidence of CHD over 10 years of follow up, despite only moderate overlap between the three definitions, which all identified individuals with insulin resistance.
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0373

Association of -1131T>C and S19W APOA5 gene variants with dyslipidemia and coronary risk among type 2 diabetic patients

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Background and Aims: Dyslipidemia in type 2 diabetes is an independent risk factor for cardiovascular diseases. Genetic factors favouring this dyslipidemia are poorly identified. We studied *APOA5* gene as a candidate because *APOA5* non-sense mutations cause familial hyperchylomicronemia and 2 common minor haplotypes are strong determinants of plasma TG variability across human populations.

Materials and Methods: We studied the impact of 2 *APOA5* gene polymorphisms, -1131T>C and S19W, on lipid profiles in 400 type 2 diabetic patients and on the occurrence of cardiovascular disease within a 5 years follow-up period in a subset of 196 patients. In group N, triglyceridemia was below the 90th percentile adjusted for age and sex (n=130). 270 patients had moderate hypertriglyceridemia (group T>90th percentile). A third group (group V, n=51) of severely hypertriglyceridemic patients with TG over 15 mM and hyperchylomicronemia was studied.

Results: -1131T>C allele was more frequent in hypertriglyceridemic diabetics (20.6% *vs.* 9.8% *vs.* 5.0%, group V *vs.* T *vs.* N, p<0.001). Hence -1131T>C carriers were hypertriglyceridemic, either moderately [OR=2.10 (1.09–4.01)] or severely [OR=4.50 (1.99–10.18)]. S19W allele was more frequent only in severely hypertriglyceridemic patients (14.0% vs. 6.5% vs. 6.1%, group V vs. T vs. N, p=0.001). Likewise S19W carriers were more at risk to undergo severe HTG [OR=3.22 (1.40–7.39)].

In group (N+T) the -1131T>C allele was associated with higher TG (+13%, p=0.034) and lower HDLc (−10%, p=0.004). S19W allele was only associated with lower HDLc (−10%, p=0.022). The risk of major coronary event was higher only in the carriers of the -1131T>C allele (OR=3.52, p=0.010 and OR=3.51, p=0.030 after adjustment)

Conclusion: Overall these results strongly suggest that *APOA5* polymorphisms may contribute to modulate dyslipidemia and cardiovascular risk in type 2 diabetes.

0374

Proinsulin/insulin, but not insulin/glucose ratio, predicts progression of carotis plaques in women

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Background and Aims: Proinsulin is increased among persons at cardiovascular risk and is also predictive for the development of diabetes. An increased secretion of proinsulin relative to insulin has been suggested as being a sign of defective proinsulin conversion associated with β-cell dysfunction. It has also been suggested that proinsulin has a more proatherogenic effect than insulin

In this prospective, population-based study we addressed whether the proinsulin/insulin or the insulin/glucose ratio predicted carotid plaque progression among non-diabetic men and women.

Materials and Methods: The study included 1886 men and 2028 women aged 25 to 82 years who were examined with B-mode high resolution ultrasound at baseline in 1994–1995 and at follow-up in 2000–2001. All plaque images were computer processed to yield a measure of plaque area in square millimetres. Proinsulin and insulin were measured at baseline. All analyses were stratified for gender

Results: Age, systolic blood pressure and tobacco smoking were associated with increments in plaque area in both genders, while cholesterol was associated with increased plaque areas in women only. After adjustment for age, baseline plaque area, waist circumference, cholesterol, HDL-cholesterol, HbA1c, glucose, blood pressure and lifestyle factors (tobacco smoking, alcohol and physical activity), the proinsulin/insulin ratio was significantly associated with follow-up plaque size in women but not in men. For a 1-SD (0.81) higher proinsulin/insulin ratio, mean plaque area was 0.92 mm^2 larger in women. Insulin/glucose ratio was not associated with changes in plaque size.

Conclusion: The proinsulin/insulin, and not the insulin/glucose ratio is an independent predictor of progression of arterial plaque size in non-diabetic women, but not in men.

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0375

Reduction of atherothrombosis for continued health (REACH) registry one-year results: cardiovascular event rates in patients with diabetes

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Background and Aims: The REACH Registry was set up to analyse cardiovascular disease (CVD) outcomes in high risk patients. We present the cardiovascular event rates in diabetic patients after one-year follow-up.

Materials and Methods: More than 68,000 outpatients aged 45 years or older with documented arterial disease or ≥3 risk factors for atherothrombosis were recruited from 44 countries all over the world, including 30,043 (44.03%) patients with diabetes. The follow-up rate after one year was 92.5%.
Results: In the diabetic patient population there were more females (39.03 *vs* 33.09%, p<0.001) and greater BMI (29.4 *vs* 27 kg/m2, p<0.001), waist circumference (101 *vs* 95 cm, p<0.001), microalbuminuria (129 *vs* 41.9 mg/d, p<0.001) and blood glucose (146 *vs* 98.4 mg/dl, p<0.001) compared with non-diabetic patients. The mean age of patients in both groups did not differ (68 years). HbA1c was not monitored. In the diabetic group 87.7% had high blood pressure, 79.4% had hypercholesterolemia and 14.2% were current smokers. At the start of the study half of the diabetic patients (51%) had a history of coronary heart disease, 25% neurovascular disease, 13% peripheral arterial disease and 30.7% had>3 risk factors. Of patients with a history of atherosclerosis, 14% had two locations (mainly coronary and neurovascular) and 1.8% had three locations, similar to non-diabetic patients. Fewer diabetic patients were treated with antiplatelet drugs (74 *vs* 82%, p<0.001) and more patients received antihypertensive treatments (93.3 *vs* 89.8%, p<0.001), but there was no difference in statin treatment (70 *vs* 68%). Further treatments included insulin (27% of patients), metformine (42%), sulfonylureas (45%), glitazones (18%), other drugs (11.5%) and diet modifications (9.8%).
After one-year follow-up, all cardiovascular event rates were higher (p<0.001) in the diabetic patient population: cardiovascular death (1.82 *vs* 1.23%), non-fatal MI (1.33 *vs* 0.97%), non-fatal stroke (1.67 *vs* 1.46%) and total CV death/MI/stroke (3.80 *vs* 3.03%). The CVD event rate was five times higher and cardiovascular death rate 7 times higher in diabetic patients with documented atherothrombosis (3 locations) compared with patients with only>3 risk factors. The non-fatal MI rate was higher in North America (1.79%) and Eastern Europe compared with Western Europe (p<0.04) and Asia (p<0.001) but non-fatal stroke rate was higher in Asia (2.27%) and Eastern Europe (2.21%) compared with North America or West Europe (1.32 and 1.86%, respectively).
Conclusion: After one-year follow-up, CVD event rate was 50% higher in diabetic patients compared with non-diabetic patients. This risk increased dramatically in patients with multiple atherothrombotic locations. The event rates and locations differed across the participating countries.
Supported by the REACH Registry is sponsored by sanofi-aventis and Bristol-Myers Squibb

PS 13

Metabolic syndrome

0376

Alanine aminotransferase and the 6-year risk of the metabolic syndrome in Caucasian men and women: the HOORN Study

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Background and Aims: The metabolic syndrome, characterised by abdominal obesity, hyperglycaemia, hypertension and dyslipidaemia, confers an enhanced risk for the development of type 2 diabetes mellitus and cardiovascular disease. Excessive deposition of fat in the liver, referred to as non-alcoholic fatty liver disease (NAFLD) is not only regarded as a risk factor but also as a feature of the metabolic syndrome. In the present study, we assessed the association between alanine aminotransferase (ALT), a marker of NAFLD, and the risk of developing the metabolic syndrome in a population-based study in Caucasian men and women.
Materials and Methods: The association of ALT with the 6-year risk of the metabolic syndrome in 1,097 subjects, aged 50–75 years, was assessed in the Hoorn Study with logistic regression analysis. Subjects with the metabolic syndrome at baseline, defined according to the Adult Treatment Panel III of the National Cholesterol Education Program, were excluded.
Results: After 6.4 (range: 4.4–8.1) years of follow-up, 226 subjects (20.6%) had developed the metabolic syndrome. The odd ratio (95% confidence interval) of metabolic syndrome, adjusted for age, sex, alcohol-intake and follow-up duration was 2.25 (1.50–3.37) for subjects in the upper tertile compared to those in the lower tertile of ALT. This association persisted after additional adjustment for all the baseline metabolic syndrome features [1.62 (1.02–2.58)]. Among the individual components of the metabolic syndrome, ALT was significantly associated only with fasting plasma glucose at follow-up.
Conclusion: ALT is associated with risk of the metabolic syndrome in a general population of middle-aged Caucasian men and women. These data indicate that NAFLD,

reflected by ALT, may herald future metabolic derangements. Further studies should elucidate the role of liver fat accumulation in the pathogenesis of metabolic syndrome and the related complications.

Table. Odds ratios (95%CI) of ALT for developing the metabolic syndrome after 6 years of follow-up

	Tertile 1	Tertile 2	Tertile 3
n	329	418	350
Model 1	1	1.43 (0.96–2.13)	2.25 (1.50–3.37)
Model 2	1	1.40 (0.90–2.18)	1.62 (1.02–2.58)

Model 1: adjusted for age, sex, alcohol-intake and follow-up duration; Model 2: Model 1+ additional adjustments for waist, glucose, HDL-cholesterol, triglycerides and systolic and diastolic blood pressure

0377

10-year trends in prevalence of the metabolic syndrome among Finnish adults

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Background and Aims: Only few studies on the trend in prevalence of the metabolic syndrome have been reported. The aim of this study is to assess the trend between 1992 and 2002 in prevalence of the metabolic syndrome defined by the National Cholesterol Education Program (NCEP) and the International Diabetes Federation (IDF) among Finnish adults.
Materials and Methods: Two cross-sectional population surveys were carried out in Finland in 1992 and 2002. A total of 2120 participants aged 45–64 years in 1992 and 2002 participants aged 45–64 years in 2002 were included in the analysis.
Results: Between 1992 and 2002, the prevalence of the metabolic syndrome was 0.9% higher based on the NCEP definition (48.9 vs 49.8%) and 1.8% higher based on the IDF definition (51.4 vs. 53.2%) in men, however, the increase was not significant (P>0.4). Decreases in high blood pressure and low high-density lipoprotein cholesterol, and increase in high fasting glucose accounted for the non-significant increase in prevalence of the metabolic syndrome in men. In women, the prevalence of the metabolic syndrome was significantly higher in 2002 than in 1992. Between 1992 and 2002, the prevalence of the metabolic syndrome among women increased from 32.2 to 36.2% based on the NCEP definition (P=0.045), and from 38.0 to 42.3% based on the IDF definition (P=0.036). Increases in abdominal obesity and high fasting glucose accounted for the increase in prevalence of the metabolic syndrome in women.
Conclusion: The prevalence of the metabolic syndrome based on the NCEP and IDF definitions has increased significantly among Finnish women during the last 10 years.
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0378

Comparison of prevalence of the metabolic syndrome and its components in population from Denmark and Japan

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Background and Aims: The Metabolic Syndrome (MS), first proposed by Raeven is a cluster of risk factors for cardiovascular disease and diabetes.
The MS definition has been proposed by various academic societies including WHO, NECP, European Group for the Study of Insulin Resistance (EGIR) and IDF.
The EGIR proposed an epidemiological approach to the MS, where insulin resistance was defined based on fasting insulin.
We analyzed the prevalence of the MS and the characteristics of the MS positive subjects from Denmark (Caucasian) and Japan (Non-Caucasian).
Materials and Methods: Out of the patients enrolled in both the Funagata study (1995–1996) and the Inter99 study (1999–2001) which are characterized as population based studies, 6703 cases in total (5686 cases in Denmark and 1017 cases in Japan) of 35–60 years of age who were analyzed. These studies were participants between 35–60 years of age who were not diagnosed with diabetes.
The MS positive subjects were defined according to the EGIR definition.
Presence of insulin resistance (upper quartile of fasting insulin level) together with at least two of the following components:
•Hyperglycemia: FPG ≥6.1 mmol/l
•Hypertension: systolic blood pressure ≥140 and/or diastolic blood pressure ≥90 and/or treatment for hypertension.
•Dyslipidemia: p-triglyceride ≥2.0 mmol/l and/or HDL cholesterol <1.0 mmol/l

•Central obesity: waist circumference ≥94 cm in men and ≥80 cm in women

We divided the eligible subjects of 35–60 years of age for analysis into 5 groups based on 5 year age ranges and compared the prevalence in men and women from the two countries. Subsequently, a comparison was made to detect what kinds of characteristics existed in MS positive subjects from the two countries. All the statistical analysis was performed using SAS System Release 8.2.

Results: The mean age of the subjects was 48.1 years in Japan and 47.3 years in Denmark.

The prevalence of the MS based on the EGIR definition among males is 2.9–9.0% in Japan and 16.3%–22.9% in Denmark, while it is, among females, 1.0–9.4% in Japan and 6.0–14.5% in Denmark.

In all the age groups, the MS prevalence of the subjects of both sexes in Denmark was 2–6 times higher than those in Japan.

Comparison of the population characteristics of the MS positive subjects disclosed that obesity-related factors such as BMI and waist circumference, as well as diastolic blood pressure, were significantly lower in Japanese whereas total cholesterol levels were significantly higher in Japanese. There were no significant differences in HDL, triglyceride and diastolic blood pressure.

Conclusion: The prevalence of the MS according to the EGIR definition in Denmark was higher than those in Japan, while differences were observed in the characteristics of the MS positive subjects who were recognized as a high risk population susceptible to the onset of both cardiovascular diseases and diabetes.It is conceivable that comparative investigation of the fundamental items comprising the MS definition among races with different genetics and life-styles plays an important role in preparation of a plausible MS definition which can obtain international consensuses.

0379

Prevalence of the metabolic syndrome in Germany assessed in a primary health care setting

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Background and Aims: The Metabolic Syndrome describes the common occurrence of central obesity, hypertension, hyperglycemia, and dyslipidemia, and is considered a major risk factor for the development of cardiovascular disease. Due to differences in currently recommended definitions (e.g. IDF, WHO, NCEP ATP III), a clear picture about its prevalence in the whole population and in subgroups is still missing. For Germany, data on the prevalence of the syndrome is rare. Aim of this study was to assess the prevalence of the Metabolic Syndrome according to the NCEP ATP III definition among Germans aged>18 years attending a general practitioner.

Material and Methods: The German-wide cross-sectional study ran two weeks in October 2005 with 1.511 randomly selected general practices from 397 out of 438 German cities and administrative districts. Blood samples were analyzed in a central laboratory, waist circumference and blood pressure assessed, data on smoking, life style, fasting status, and sociodemographic characteristics collected. Only pregnant or breast feeding women and patients with severe illness were excluded from the study. Non-responders information about gender, age and reasons for nonparticipation were collected. Since food intake has an impact on glucose and triglyceride levels, a two-stage approach was taken for blood sampling. First, all patients on a predetermined examination day were included, yielding about 35.000 random blood samples. In case the findings concerning fasting serum glucose or triglycerides were ambiguous due to a meal in the previous 12 hours, the patient was asked to come for a second appointment to give a fasting blood sample. Furthermore, a random sample (30%) of participants were asked to come for a second fasting blood sample, regardless of the first test result. These data provide further useful information, e.g. estimation of intraindividual variations.

Results: In total, 35.869 patients were included (age range: 18–99, women 61,1%). More than 50.000 blood samples were taken. Fasting blood samples are available for 49% of the participants (42% fasting>12 hours, 7% fasting 8–11 hours). For another 49%, random blood glucose and triglyceride samples as well as data on time and type of last meal are available. Prevalence of hypertension was 58,2% (95%CI: 57,7–58,7), abdominal obesity 39,5% (39,0–40,0), hyperglycemia 17,0% (17,3–18,1), triglyceridemia 16,9% (16,5–17,4) and low HDL-C 13,2% (12,9–13,6). The prevalence of the Metabolic Syndrome according to the NCEP ATP III definition was 19,8% (95% CI: 19,4–20,2), with women aged>60 years showing the highest prevalence rate (32,8%; men: 31,5%), women <45 years showing the lowest rate (6,6%; men: 10,6%).

Conclusion: The findings of this study show a high prevalence of the Metabolic Syndrome in the German

population attending a general practitioner. Prevalence rates increase with age in both genders.
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0380

Gender differences in metabolic features of type 2 diabetes in the AGES-Reykjavik Study

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Background and Aims: A population based study with data gathered in late life as well as midlife is used to compare the metabolic features of type 2 diabetes (T2D) in the genders, diagnosed in midlife vs. later life, and this related to cardiovascular risk levels.
Materials and Methods: Data are presented from 2279 participants (mean age 76 years) attending the population based AGES-Reykjavik Study and recruited over the period 2002–2004. T2D was identified by self-report of diabetes, use of diabetes medication, or fasting S-glucose of ≥7 mmol/L. The T2D group is subdivided into three groups, two according to age at T2D diagnosis into midlife onset (age 40–66 years) and onset in later life (age ≥67 years) and a third group diagnosed when recruited. Features of glucometabolic control and cardiovascular risk characteristics of the genders are compared.
Results: Of the 1313 women participating 9.2% were diagnosed with T2D and 14.8% of the 966 men. Mean age at diagnosis was 68 years. In midlife onset T2D, 48% report a positive family history, compared to 34% in the late onset group, and 19% of controls. Midlife BMI measured at the mean age of 52 years was greatest in those with midlife onset T2D. However, the largest increase in BMI with age was seen in the late onset group, especially in women. Men in midlife had a higher mean BMI than women but that is reversed later in life. Diabetic women as a whole had higher systolic blood pressure than diabetic men in spite of more intensive hypertensive medication. They also showed considerably greater increase in systolic BP from midlife values than men. Urinary albumin creatinine ratio is significantly higher in diabetic men than in women indicating greater prevalence of kidney damage in men. 90% of diabetic men receive medication affecting glucose metabolism but just over 50% of the women. Still the glucometabolic profile of diabetic men is worse than in women, showing both higher fasting glucose and higher HbA1c. Total and LDL cholesterol is higher in all subgroups of women than in corresponding groups of men, partly explained by statins being more commonly prescribed for men in general than women. Self reported overall prevalence of coronary events (MI, GABG, PCI) in women is 11.5% and 29.7% in men. The highest prevalence is in midlife onset T2D in both genders, but cases are few in number and the difference does not reach statistical significance.
Conclusion: Prevalence of T2D is higher in men than women. In both genders, midlife onset of T2D had a worse metabolic profile in old age than persons with late onset T2D. Midlife onset T2D was also more associated with positive family history and higher BMI. Diabetic women had higher systolic pressure, and higher LDL than diabetic men, but a more favourable urinary albumin creatinine ratio than men. This suggests that diabetes in old age although a heterogeneous disease, dependent on family history, duration of disease, and weight history, may present different effects on the genders as reflected in unfavourable cardiovascular risk levels in diabetic women.

0381

National Cholesterol Educational Program (NCEP) and International Diabetes Federation (IDF) criteria for metabolic syndrome in an Italian cohort. Results from the FIBAR Study

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Background and Aims: The new IDF criteria for Metabolic Syndrome (MS), in comparison with NCEP, produces different changes in estimates of prevalence in several populations. Few data are available in Caucasian non-diabetic subjects. Aim of this study is to evaluate the prevalence of NCEP- and IDF-defined MS in a cohort of Caucasians subjects.
Materials and Methods: A sample of 2945 individuals (aged 55.2±11.5 years, BMI 26.0±4.2(kg/m^2) with no history for diabetes, resident in Florence and Bagno a Ripoli municipalities enrolled in a screening program for diabetes. Association of different definitions of MS with glucose intolerance (120-min glucose 7.8 mmol/l after a 75 g-oral glucose load) and hyperuricaemia (uric acid>0.38 mmol/l) was also assessed.
Results: The diagnosis of metabolic syndrome was made in 489 (16.6%), 680 (23.1%) and 875 (29.7%) subjects with NCEP, NCEP revised and IDF definitions, respectively. The prevalence of IDF-defined MS was higher than NCEP-MS

through all age ranges; among those individuals aged>60 years, the prevalence of IDF-MS reached 52.8% (vs. 33.1% for NCEP-MS). The prevalence of NCEP-defined MS in lean (BMI <25 kg/m^2), overweight and obese subjects was 3.2%, 19.8% and 45.2%, while applying IDF criteria was 8.9%, 36.8% and 62.4%, respectively. The proportion of individuals with pathological waist circumference according to NCEP criteria in lean, overweight and obese subjects was 3.8%, 36.7%, and 89.2%, respectively, while the corresponding figures using IDF criteria for waist circumference (European population cut-points) were 36.7%, 89.2%, and 99.2%, respectively. Both NCEP- and IDF-MS criteria were associated with glucose intolerance and hyperuricaemia. Individuals fulfilling IDF, but not NCEP criteria for the diagnosis of MS, showed a prevalence of glucose intolerance (22.7%) significantly ($p<0.05$) lower than those fulfilling NCEP criteria only (31.6%) or both sets of criteria (31.8%).

Conclusion: In Caucasian subjects without known diabetes, IDF criteria produce a relevant increase in estimates of prevalence of MS, particularly in older and lean subjects individuals, when compared with NCEP criteria. NCEP-MS seems to be more effective than IDF-MS in the identification of glucose intolerant subjects.

0382

Low prevalence of the metabolic syndrome in diabetic African men by ATPIII and IDF definitions: a study in African, white and south Asian men and women

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Background and Aims: Type 2 diabetes is associated with a high prevalence of the Metabolic Syndrome (MS) in most populations. MS severity appears to be linked to coronary heart disease, which is rare in diabetic Africans. We have compared the prevalence and severity of the MS, defined by the ATPIII (ATPIII-MS) or IDF (IDF-MS) criteria, in African (A), white (W) and South Asian (S–A) men and women with diabetes, the latter populations at high risk for CHD.

Materials and Methods: The presence of the MS was assessed in 895 subjects with stable Type 2 diabetes: (A, 500; W, 254; S–A, 141) using ATPIII criteria (waist>102 cm for men; >88 cm for women) and IDF criteria (waist: men, >94 cm for A and W;>90 cm for S–A; women,>88 cm); other criteria were the same. Severe MS was defined as diabetes plus 3 or 4 additional elements of the MS.

Results: In **men** ATPIII-MS was found in 55.0% A, vs 74.1% W ($p<0.001$) and 67.8% S–A ($p=0.11$); severe MS in 28.7%, vs 51.1% ($p<0.001$) and 33.5% ($p=0.27$) respectively. IDF–MS was present in 65.1%, 82.2% and 89.8% ($p<0.001$), and severe MS in 33.5%, 56,3% and 59.3% in A, W ans S–A respectively ($p<0.001$). Both A and S–A had smaller waists than W ($p<0.001$); triglycerides were lowest in A ($p<0.001$). In the diabetic **women** the prevalence of both ATPIII–MS and IDF–MS was similar in each ethnic group (82.5–93.9%); but severe MS by both definitions was lowest in the A group ($P<0.007$), explained by lower triglyceride levels ($p<0.001$).

Conclusion: The prevalence of MS and severe MS, by both definitions, was lowest in the diabetic African men, in whom a waist circumference of 90 cm may be appropriate for the IDF–MS, as in S–A men. In diabetic Africans the lower prevalence of the MS in men, and of severe MS in both sexes, might contribute to the previously observed low prevalence of CHD in this population.

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0383

Are the patients with metabolic syndrome as defined by International Diabetes Federation criteria more insulin resistant than NCEP-ATP III criteria?

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Background and Aims: We analyzed how well International Diabetes Federation (IDF) definition of the metabolic syndrome reflect insulin resistance status in middle-aged adults in Korea and compared it with NCEP-ATP III criteria.

Materials and Methods: This study was performed from February to April, 2003-2005 in adults over 40 years living in the Chungju city, South Korea, selected by the stratified random cluster sampling. We analyzed the data from total 8,395 subjects (3,436 men and 4,959 women) who completed the survey consisting of anthropometric measurement and blood chemistry. Metabolic syndrome was defined as by 2 different definitions, IDF and NCEP-ATP III. We analyzed how well these two criteria reflect insulin resistant status when we define the highest quartile of HOMA-IR in whole study population as insulin resistant (IR) group using sensitivity, positive likelihood ratio, negative predictive value and area under the curve (AUC) of ROC for detecting IR group.

Results: The age-adjusted prevalence of the metabolic syndrome using IDF definition was 30.2%. According to NCEP-ATP III criteria, 34.5% of the study subjects had metabolic syndrome. There was no significant difference

between the AUC value of IDF and NCEP-ATP III criteria in total study subjects (p=0.569). However, IDF definition did better reflect insulin resistance in non-diabetic population significantly (p=0.001 in total subjects, p<0.001 in women) except in men (p=0.668). Furthermore, positive likelihood ratio by IDF definition was somewhat higher than those by NCEP-ATP III criteria.
Conclusion: From our result, new IDF definition was more specific, but less sensitive than that of NCEP-ATP III criteria for detecting insulin resistance group. Overall diagnostic accuracy for insulin resistance group by IDF definition was somewhat superior than that by NCEP-ATP III criteria, but its difference was trivial.

0384

Comparison of the prevalence of the metabolic syndrome in women with prior gestational diabetes mellitus using two definitions

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Background and Aims: To estimate the prevalence of the metabolic syndrome (SM) in women with previous gestational diabetes mellitus using the Adult Treatment Panel III (ATP-III) and the International Diabetes Federation (IDF) definitions and to analyse the differences between both classifications.
Materials and Methods: We studied 1080 women (mean age: 33±4 years; mean BMI: 25.6±4.7) with GDM diagnosed between 1992 and 2004, who attended the postpartum follow-up visit (3–6 months postpartum). Assessment included: measurement of weight, height, waist circumference, blood pressure, fasting glucose level and lipid profile.
Results: The prevalence of the MS was 9.3% according to the ATP-III definition and 17.4% according to the IDF definition. Among all women, 8.7% had the MS under the two definitions, 0.6% had the MS under the ATP-III definition but not the IDF definition (due to the absence of abdominal obesity) and 8.7% had the MS under the IDF definition but not the ATP-III definition (due to the lower fasting glucose criteria in 17,02% of cases, to the lower abdominal circumference criteria in 13,82% of cases and to both criteria in 69,15% of cases). The prevalence of abdominal obesity was 19.2% using the ATP-III definition and 48.1% using the IDF definition. The prevalence of high fasting glucose was 8.7% using the ATP-III definition and 27,4% using the IDF definition. Among all women, 41.6% under the ATP-III definition and 26.5% under the IDF definition had all five criteria below the cut-off value.
Conclusion: The prevalence of the MS in women with prior GDM using the IDF definition is almost two times higher than using the ATP-III definition. Only 26.5% of women with prior GDM have no risk factors under the IDF definition.

0385

Identifying metabolic syndrome in type 2 diabetes: is it needed?

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Background and Aims: The International Diabetes Federation (IDF) recently proposed a new world-wide definition of metabolic syndrome (MetS) emphasising the importance of central adiposity (as defined by ethnic-specific waist circumference) as a prerequisite to identify this condition. This differs from NCEP-ATPIII criteria which does not emphasise central adiposity as a prerequisite condition. These criteria function as clinical tools to identify patients at risk of developing cardiovascular disease and type 2 diabetes (T2DM). In T2DM, MetS is associated with higher incidence of cardiovascular disease. A recent study compared IDF with NCEP-ATPIII criteria in non-diabetic patients and demonstrated higher prevalence of MetS with IDF criteria (~40%). The aim of this study is to determine the prevalence of MetS with IDF and NCEP-ATPIII criteria in T2DM and its relevance in clinical practice.
Materials and Methods: Patients who attended hospital diabetes clinics were recruited. The following data were collected: age, sex, diabetes duration, body mass index, waist circumference, blood pressure, HDL cholesterol, triglyceride and HbA1c. IDF and NCEP-ATPIII criteria were used to define MetS.
Results: 252 patients were recruited. IDF and NCEP-ATPIII criteria identified ~93% of T2DM patients to have MetS with good concordance between these two criteria (κ=0.71, p<0.0001). Approximately 92% of patients have MetS by both criteria. Prevalence of MetS was higher in women (~97% with both criteria) who had similar degree of central obesity as men (waist circumference; women vs men, 114.8 vs 114.0 cm, p=NS). Among young T2DM patients (<40 yrs), MetS prevalence was ~70%. After the age of 40, prevalence of MetS was higher and remained static (~95% with both criteria) across different age groups. MetS was associated with higher prevalence of ischaemic heart disease (IDF; MetS vs no Mets, 33.1 vs 6.3%, p=0.025 and NCEP-ATPIII; MetS vs no MetS, 33.2 vs 5.9%, p=0.019) and cardiovascular disease (IDF; MetS vs no MetS, 41.5 vs 6.3%, p=0.005 and NCEP-ATPIII; MetS vs no MetS, 41.7 vs 5.9%, p=0.003).

Conclusion: IDF and NCEP-ATPIII criteria similarly identified patients with MetS in T2DM. Given its extremely high prevalence, these data support the concept of T2DM as a high risk condition and also suggest that identification tool for MetS is not needed in the clinical management of T2DM.

PS 14

Obesity, abdominal obesity and insulin resistance

0386

Is there any agreement between HOMA 1 and HOMA 2 models?

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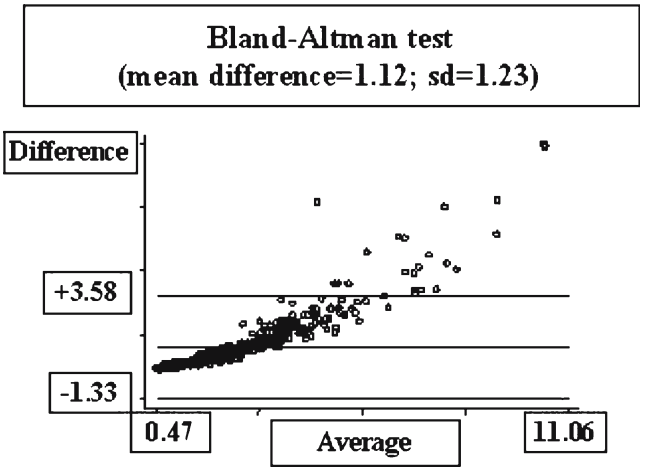
Background and Aims: To determine the cut off of HOMA 1 and HOMA 2 considering Impaired Glucose Tolerance and type 2 Diabetes as independent variable.
To evaluate concordance between HOMA 1 and HOMA 2 models.
Materials and Methods: Six hundred twenty one patients, age 43.1±12.2 years old, male 313 (50.4%), female 308 (49.6%), BMI 32.8±6.1; euthyroids, without previous diagnosis of type 2 Diabetes (DM2) or Impaired Glucose Tolerance (IGT) or treated with metformin, thiazolidinediones, oral hypoglycemiant drugs, sibutramina and orlistat were studied.
Patients with fasting insulinemia ≤2.9 mU/ml or ≥43.8 mU/ml were excluded by HOMA 2 model's limitations.
Insulinemia was made by electrochemiluminiscense.
Diagnosis of IGT or DM2 were made with WHO criteria.
HOMA 1 model was calculated by original model from Matthieus et al.(1985) and HOMA 2 model by homa 2 calculator, Oxford.
The cut off of HOMA models were calculated by ROC curve, considering DM2 and IGT as independent variable.
Statistical analysis were made through receiver operating curve (ROC), sensitivity, especificity, Bland & Altman test and Kappa (κ) coefficient (significative: $p<0.05$).
Results: Patients were classified as IGT n=39 (6.3%) and DM2 n=45 (7.25%).
The cut off of HOMA 1 in DM2 and IGT was 3.04 (AUC: 0.845, $p<0.001$), sensitivity of 0.80 and especificity of 0.77, and the cut off of HOMA 2 was 2.05 (AUC: 0.803, $p<0.001$), sensitivity and especificity of 0.73 and 0.77 respectively.

Table 1: Cut off of HOMA 1 and HOMA 2 in Impaired Glucose Tolerance and type 2 Diabetes

Dependent variable	Independent variable	Cutt of	AUC	Significance	Sensitivity	Especificity
HOMA 1	DM2+IGT	3.04	0.845	p<0.001	0.80	0.77
HOMA 2	DM2+IGT	2.05	0.803	p<0.001	0.73	0.77

The mean difference between HOMA 1 and HOMA 2 was 1.12±1.23 by Bland & Altman test (graphic 1).



The κ coefficient (concordance among HOMA 1 and HOMA 2 models) was 0.43, $p<0.05$. The agreement between different ranks of HOMA 1 and HOMA 2 can be found in table 2.

Table 2: Concordance between different ranks of HOMA 1 and HOMA 2

	HOMA 2:≤0.99	*HOMA 2:* 1.0–1.99	*HOMA 2:* 2.0–2.99	*HOMA 2:*≥3.0
HOMA 1: ≤0.99	**80**	0	0	0
HOMA 1: 1.0–1.99	112	**112**	0	0
HOMA 1: 2.0–2.99	0	115	**7**	0
HOMA 1: ≥3.0	2	11	115	**67**

The differences of cut off and concordance between HOMA 1 and HOMA 2 remain when they were adjusted by sex.

Conclusion: According to the selected independent variable (DM2 and IGT) the cut off of HOMA 1 and HOMA 2 models were 3.04 and 2.05 respectively.
We found a disagreement between HOMA 1 and HOMA 2 by Bland & Altman test and κ coefficient.
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0387

The impact of physical activity on estimates of insulin sensitivity and glucose-induced insulin release – the INTER99 study

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Background and Aims: Deterioration of glucose tolerance is caused by a combination of increasing insulin resistance and impaired insulin secretion and can be postponed/prevented by increased physical activity. The aim of this study was to analyse whether the level of physical activity is associated to insulin resistance.
Materials and Methods: The analysis is based on cross-sectional data from the population-based Inter99 study (N=4,540, aged 30–60 yrs). The history of physical activity (at work and leisure time) was assessed by questionnaires and categorised into 4 levels. All individuals underwent an OGTT including measurement of plasma glucose and serum insulin at time 0, 30 and 120 min. Insulin resistance was estimated by HOMAir and the Gutt index. Oral glucose-induced insulin release was estimated by the insulinogenic index, IGI. The impact of physical activity on insulin sensitivity and insulin release was assessed using multiple linear regression analyses adjusted for sex, age and BMI.
Results: HOMAir was positively and the Gutt index was inversely associated (i.e. both estimates of insulin sensitivity were inversely associated) – and IGI was positively associated with decreasing level of physical activity.

Table1: Estimates on relative change (95% CI) in outcome for the relationship between physical activity at work and leisure time and HOMAir, the Gutt index and IGI.

	HOMAir, ($mmol \cdot mIU \cdot l^{-2}$), †,a	Gutt index, ($mg \cdot l^2 \cdot mmol^{-1} \cdot mIU^{-1} \cdot min^{-1}$), †,a	IGI, ($pmol \cdot mmol^{-1}$), †,a
Stronger/heavy physical activity	0.81 (0.76–0.87)	1.20 (1.15–1.25)	0.84 (0.78–0.92)
Intermediate physical activity	0.88 (0.84–0.92)	1.11 (1.08–1.15)	0.90 (0.85–0.96)
Light physical activity	0.93 (0.90–0.96)	1.07 (1.04–1.10)	0.94 (0.89–0.98)
Physical passivity	1	1	1

†) The models used log-transformed dependent variables.
a) $p<0.0001$.

Conclusion: Decreased levels of physical activity are positively associated with estimates of insulin resistance and positively associated with an estimate of insulin release after an oral glucose load.
Supported by the Danish Medical Research Council, the Danish Center for Evaluation and Health Technology Assessment, Novo Nordisk, Copenhagen County, the Danish Heart Foundation, the Danish Diabetes Association, the Danish Pharmaceutical Association

0388

WITHDRAWN

0389

Waist circumference and diabetes prevalence in European primary care patients: the IDEA study

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Background and Aims: IDEA (the International Day for the Evaluation of Abdominal obesity) was an international, non interventional, cross-sectional study designed to estimate the prevalence of abdominal obesity (measured by waist circumference; WC) and its association with cardiometabolic risk factors in 168 159 patients attending primary care physician (PCP) offices on two predefined half days in 63 countries worldwide. The aim of this analysis was to assess the prevalence of diabetes in 3 European regions and describe its relationship with anthropometric parameters (WC, and body mass index [BMI]).
Materials and Methods: WC, weight and height were measured by the PCPs; demographic data and the presence of diabetes were recorded. Data from 3 European regions were analysed (NW Europe: Austria, Belgium, Denmark, Finland, France, Germany, Ireland, The Netherlands, Norway, Sweden, Switzerland; S Europe: Greece, Italy, Portugal, Spain, Turkey; E Europe: Bulgaria, Czech Republic, Estonia, Hungary, Latvia, Lithuania, Poland, Russia, Slovakia, Slovenia, Ukraine). For each gender, diabetes prevalence was age-standardised with the entire European population as reference. Odds ratios (ORs) and 95% confidence intervals (CIs) for the risk of having diabetes were estimated from multiple logistic regression models after adjusting for age.
Results: See table.

Conclusions: In a primary care population, the prevalence of diabetes was higher in Southern Europe, compared with North Western and Eastern Europe. Across regions, the prevalence of diabetes was higher in men than women. Irrespective of geographical region, WC was strongly associated with diabetes independently from age and BMI, and this relationship was even stronger in women.

	Men			Women		
	NW Europe	S Europe	E Europe	NW Europe	S Europe	E Europe
	(n=12 796)	(n=13 465)	(n=11 176)	(n=16 786)	(n=17 824)	(n=19 199)
Mean (SD) age, years	52.3 (16.1)	53.8 (15.9)	49.5 (16.0)	51.2 (16.5)	52.4 (15.8)	50.7 (15.9)
Mean (SD) WC, cm	97.8 (13.5)	99.4 (12.9)	96.9 (13.7)	88.3 (14.8)	91.3 (14.7)	89.7 (15.7)
Mean (SD) BMI, kg/m^2	27.2 (4.6)	28.2 (4.5)	27.5 (4.8)	26.4 (5.6)	27.9 (5.6)	27.6 (6.0)
BMI ≥30 kg/m^2, %	23.0	30.2	27.5	22.7	31.7	32.4
Prevalence of diabetes, %	12.8	14.5	12.4	8.8	11.5	10.7
WC, diabetes OR (95% CI)*	1.76 (1.67–1.86)	1.45 (1.38–1.53)	1.83 (1.72–1.95)	2.17 (2.05–2.29)	1.78 (1.69–1.87)	1.98 (1.88–2.09)
WC, diabetes OR (95% CI)* (adjusted for BMI)	1.35 (1.24–1.48)	1.29 (1.20–1.39)	1.46 (1.32–1.63)	1.72 (1.58–1.88)	1.45 (1.35–1.56)	1.59 (1.46–1.73)

*Odds ratio (95% CI) for diabetes for a 1 SD increase in WC. SD, standard deviation.

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0390

Change in waist circumference and metabolic consequences over 9 years: the D.E.S.I.R. Study

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Background and Aims: The long term effect on metabolic parameters, of gaining or loosing abdominal adiposity has been little analysed in longitudinal studies.
Materials and Methods: 1863 men and 1938 women, aged 30–65 years at inclusion in the French D.E.S.I.R. cohort (Data from an Epidemiological Study on the Insulin Resistance Syndrome) were studied for changes in metabolic syndrome parameters over nine years.
Results: At baseline, 8% of men and 13% of women had NCEP-ATP III defined abdominal adiposity: waist >102/88 cm (men/women) and 31% of men and 33% of women had IDF defined abdominal adiposity: waist >94/80 cm (men/women); at 9 years, percentages were 16% and 25% for NCEP-ATP III and 43% and 50% for IDF. Over this period, 25% of the men and 34% of the women increased their waist by more than 7 cm, while 14% and 13% lost more than 2 cm. In subjects with a 7 cm or more increase in waist circumference, in comparison to those with a stable waist (±2 cm), the age-adjusted odds ratios, for ***incident*** syndrome abnormalities are tabulated below. These results were little attenuated after adjusting on BMI.
Conclusion: Increasing waist circumferences had similar deleterious metabolic effects in men and women, which were accentuated when combined together into the metabolic syndrome: odds-ratios were high in both men and women whose waist circumferences increased by more than 7 cm over the 9 years, partly because the syndrome includes high waist circumference as a criterion.
Odds ratios, age adjusted, for incident abnormalities

	Men	Women
hyperglycaemia (>6.1 mmol/l)	1.7 (1.0–2.8)	2.7 (1.4–5.0)
hyperglycaemia (>5.6 mmol/l)	1.6 (1.0–2.4)	2.4 (1.5–3.6)
high arterial blood pressure (SBP/DBP>130/85 mmHg)	1.5 (0.9–2.4)	1.5 (1.0–2.2)
hypo-HDL-cholesterolaemia (<1.03/1.29 mmol/l m/w)	2.0 (1.1–3.4)	1.9 (1.2–2.8)
hypertriglyceridaemia (>1.7 mmol/l)	2.3 (1.5–3.5)	2.2 (1.4–3.6)
NCEP-ATP III metabolic syndrome	6.6 (3.9–11.3)	4.5 (2.6–7.4)
IDF metabolic syndrome	5.7 (3.9–8.4)	10.8 (6.6–17.4)

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0391

Optimal cut off value of waist circumference reflecting insulin resistance as diagnostic criteria of metabolic syndrome in nondiabetic Korean population: from CMS (Chungju Metabolic Syndrome) study

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Background and Aims: In 2005, the International Diabetes Federation proposed a new worldwide definition of

metabolic syndrome that abdominal obesity is included as a prerequisite risk factor. Various cut-points of waist circumference that represent abdominal obesity were provided from different countries or ethnic groups. Insulin resistance plays the major role in the pathophysiology of metabolic syndrome, and a positive association between central obesity and insulin resistance has been consistently observed in many studies. So determining the cut off level of abdominal obesity to best predict insulin resistance will be more relevant to the explanation of pathophysiology of metabolic syndrome. We aimed to determine the cut off value of waist circumference with respect to its ability to reflect insulin resistance in nondiabetic Korean population.
Materials and Methods: The CMS (Chungju metabolic syndrome) study is a community-based observational study of metabolic syndrome. 8,395 subjects aged over 40 years old were selected by stratified random cluster sampling between the year of 2002 and 2004, and 6,078 nondiabetic subjects (2,434 men and 3,644 women) without missing data about insulin, glucose, and other covariates were analyzed. Insulin resistant individuals were defined as the subjects with the highest quartile value of HOMA-IR which was determined among non-diabetic population. The associations of the waist circumference with insulin resistance were analyzed using multiple logistic regression analysis. The optimal sensitivity and specificity for predicting insulin resistance were the values producing maximum sums from receiver operating characteristic (ROC) curve in men and women separately.
Results: After controlling for age, systolic blood pressure, diastolic blood pressure, triglyceride, HDL cholesterol, and body mass index, the association was significant in the category of>85–90 cm (OR 2.19, 95%CI 1.07–4.51 form men; OR 1.89, 95%CI 1.25–2.87 form women) when the category of <70 cm of WC was taken as a reference, and the statistically significant association was also observed over the categories of>85–90 cm. And the cut off value of waist circumference for ROC analysis was 84.4 cm for men (70.7% sensitivity and 58.3% specificity), and 84.7 cm for women (55.4% sensitivity and 70.2% specificity).
Conclusion: Cutoff value for waist circumference predicting insulin resistance was considered to be 85 cm for men and women, respectively, suggesting that Asian–Pacific criteria of abdominal obesity might not be applicable for Korea.

0392

Serum uric acid is not a predictor of cardiovascular mortality in type 2 diabetes: the Casale Monferrato Study

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Background and Aims: Studies have indicated that serum uric acid, the end product of purine catabolism, is associated with hypertension, obesity, hyperinsulinemia, dyslipidemia, suggesting that it could be a component of metabolic syndrome. Whether serum uric acid is an independent predictor of cardiovascular mortality in type 2 diabetes is actually unknown. Aims of this work were to assess in a population-based cohort of type 2 diabetes variables associated with serum uric acid and its role as an independent predictor of cardiovascular mortality, independently on other risk factors.
Materials and Methods: The study base was the population-based cohort of 1565 diabetic subjects (median age 68.9 yrs) of the Casale Monferrato Study. The predictive roles of serum uric acid on 11-yrs all-cause and cardiovascular mortality (ICD-9 codes 390–459) was assessed by using multivariate Cox proportional hazards modelling, adjusting for classical risk factor (age, sex, hypertension, smoking, CHD), novel risk factors (AER, fibrinogen), HbA_1C cumulative average and referring physicians.
Results: Levels of serum uric acid were higher in males (5.55 vs 5.29 mg/dl, p=0.003), elderly (5.49 vs 5.34 mg/dl, p=0.08), obese people (p<0.0001), those with diabetic nephropathy (5.56 mg/dl for AER≥20 μg/min vs 5.25 mg/dl for AER <20 μg/min), hypertension (5.49 vs 4.93 mg/dl, p<0.0001), higher levels of serum creatinine (>1.16 mg/dl) vs lower (<0.91 mg/dl) (6.22 vs 4.75, p<0.001) and in people with the metabolic syndrome (WHO criteria) (5.57 vs 4.87 mg/dl, p<0.0001); 685 death were identified in 10890.2 person–year of observations. All cause mortality was 63/1000 (95% CI 59–68) and cardiovascular mortality was 31/1000 (95% CI 28–35). In univariate analysis, mortality risk increased with increasing levels of serum uric acid (HR 1.36, 95% CI 1.00–1.85, in the upper vs lower quartile). HRs, however, were no more significant after adjustment for age and sex (HR 1.18, 95% CI 0.86–1.61), for the above and for hypertension, apoB/apoA1 ratio, and smoking (HR 1.00, 95% CI 0.72–1.39), for the above and for AER, fibrinogen and individual cumulative HbA1c (HR 0.89, 95% CI 0.63–1.24).
Conclusion: Our population-based study indicates that in Mediterranean diabetic population, serum uric acid is associ-

ated with component of the metabolic syndrome but it is not an independent risk factor of cardiovascular mortality.

0393

Genetic and environmental factors that modulate serum adiponectin concentrations in diabetic and non-diabetic participants in the PREDIMED trial

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Background and Aims: Many studies have reported that low plasma adiponectin concentrations associate closely higher adiposity, diabetes, hypertension and dyslipidaemia. Although evidence indicates that adiponectin is anti-inflammatory, some studies in diabetic patients have demonstrated that adiponectin is associated with impaired renal function and may be enhanced as a physiological counter-regulatory response to mitigate endothelial damage. The aim of our study was to compare adiponectin levels between diabetic and non diabetic subjects and to study genetic and environmental factors that may be associated with adiponectin concentrations in a high-cardiovascular risk population.
Materials and Methods: We conducted a cross-sectional study in 266 (110 men and 156 women) high-cardiovascular risk patients participating in the PREDIMED (PREvencion con DIeta MEditerranea) trial. They were men (age: 55–80) and women (age: 60–80) free of CHD but with either diabetes or at least three major cardiovascular risk factors (smoking, high blood pressure (HBP), high LDL-C, low HDL-C, overweight, and family history of premature CHD), recruited in the Valencia region (Spain). Adiponectin, anthropometric, clinical, biochemical, and life style variables were determined. All subjects were genotyped for the c.331 T>C mutation (Y111H) in exon 3.
Results: Prevalence of diabetes in this sample was 47%. Adiponectin concentrations were statistically higher in women than in men (11±5.1 vs 6.9±3.3 μg/mL; p<0.001). Moreover adiponectin was statistically significant lower in diabetic than in non-diabetic patients (7.8±4.6 vs 11.4±4.8 μg/mL; p<0.001). We did not find differences in adiponectin between obese and non-obese subjects. Although plasma adiponectin was slightly higher in non-smokers than in smokers, differences did not reach the statistical significance. Adiponectin was not associated with hypertension (P=0.214). We found statistically significant correlations between adiponectin and HDL-C (r=0.36; P<0,001), triglycerides (r=−0.28; P<0.001), glucose (r=−0.28; PC polymorphism was very low (96.1% TT, 3.9% TC and 0% CC). Although prevalence of the variant allele was two times lower in diabetics (2.6%) than in non-diabetics (5.2%), results were not statistical significance (P=0.179). Similarly, plasma adiponectin concentrations were non-significantly (P=0.29) higher in carriers of the variant allele (11.7±7.3 μg/mL) than in TT patients (9.7±4.9 μg/mL).
Conclusion: In this high-cardiovascular risk population, diabetes and gender were the factors most related to adiponectin concentrations
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0394

Metabolic syndrome and adipocytokines in Japanese children aged 12–13 years old

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Background and Aims: Obesity is a serious health issue for children around the world. In this study, we sought to determine the prevalence of metabolic syndrome (MetS) and the levels of biochemical markers including adipocytokines of children with/without MetS in Japanese children aged 12–13 years old.
Materials and Methods: As a population-based health promotion plan, 291 (138 boys and 152 girls) out of a total of 307 school children (participation rate: 94.8%) in the first grade of all 3 junior high schools in Ina-town, Saitama, participated in this study in 2004. MetS in children was defined as having a waist circumference≥90 percentile of this group (83 cm for boys and 75 cm for the girls) plus any two of the following four factors: raised TG level (≥150 mg/dl), reduced HDL cholesterol (<40 mg/dl), raised blood pressure (SBP≥130 mmHg), or raised plasma glucose(≥100 mg/dl). The levels of the biochemical markers leptin, adiponectin, resistin, high-sensitivity C reactive protein (hCRP), insulin (IRI), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and low density lipoprotein cholesterol (LDL) of children with MetS were compared to the levels of those without MetS. Statistical significance was examined by the Mann-Whitney test using the SPSS program. The study protocol was approved by two independent institutional review boards. Written informed consent was obtained from all participants and their parents.
Results: Six boys and one girl met MetS criteria, giving prevalence rates of MetS of 4.3% for boys and 0.7% for girls. Children with MetS have significantly higher median levels (intraquatile range) compared with those without MetS for leptin (MetS: 12.8 (10.6–14.9), non MetS: 3.4 (2.1–5.4) ng/ml, p<0.001), hCRP (MetS: 0.67 (0.37–1.5), non MetS: 0.11 (0.07–0.26) mg/dl, p=0.001), IRI (MetS: 37.6 (32.0–65.7), non MetS: 10.2 (6.0–15.7) μU/ml,

p<0.001), AST (MetS: 29 (20–44), non MetS: 20 (18–23) IU/l, p=0.015) and ALT (MetS: 51 (30–70), non MetS: 12 (10–14) IU/l, p<0.001). The level of adiponectin in children with MetS tended to be lower than those without MetS (MetS: 6.1 (5.6–10.9), non MetS: 9.1 (7.5–11.6) μg/ml), however the difference did not reach statistical significance (p=0.051). The levels of resistin (MetS: 7.1 (5.8–10.6), non MetS: 6.5 (4.5–8.9) ng/ml, p=0.227) and LDL (MetS: 87 (85–131), non MetS: 91 (79–107) mg/dl, p=0.348) did not differ between children with MetS and those without MetS.
Conclusion: The current study indicated that 2.4% of the Japanese children aged 12–13 years old had MetS, characterized by elevated leptin, hCRP, IRI, AST and ALT. It is indicated that lifestyle intervention should be initiated at least around puberty.
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PS 15

Treatment, metabolic control and outcome in diabetes

0395

Utilisation of antihyperglycaemic drugs in ten European countries: different developments and different levels
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Aim: To compare secular trends and developments of the utilisation of antihyperglycæmic drugs (AHGD) in ten European countries.
Methods: Data on the yearly (1994–2003) utilisation of insulin and oral AHGD were collected from public registers in Denmark, Finland, Norway, Sweden, Belgium, England, Germany, Italy, Portugal and Spain, and were expressed in Defined Daily Doses per 1000 inhabitants per day.
Results: Total AGHD utilisation increased in each of the ten countries but at different rates and levels. Insulin utilisation doubled in England and Germany but hardly changed in Belgium, Portugal and Italy. Sulphonylurea utilisation doubled in Spain, England and Denmark but was reduced in Germany and Sweden. Metformin utilisation increased strongly everywhere. In 2003, there were two- to three-fold differences in AHGD utilisation even between neighbour countries, e.g. Denmark vs Finland, Denmark vs Sweden, Belgium vs Germany and Portugal vs Spain. In Finland, there were more users of both insulin (+120%) and oral AHGD (+80%) than in Denmark, and also the daily oral AHGD doses were 20–25% higher. In Denmark and Sweden, AHGD utilisation was equal in subjects <45 but in those>45 both insulin and oral AHGD utilisation were twice as high in Sweden.
Conclusion: The ubiquitous increase in AHGD utilisation, especially of metformin, seems rational, considering the increasing prevalence of type 2 diabetes and the results of the UKPDS. However, the large differences even between neighbour countries appear less rational and suggest different habits and attitudes concerning screening and management of type 2 diabetes.
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0396

The risk of cancer with antidiabetic agents: evidence from clinical trial and observational data
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Background and Aims: Preliminary data suggest a beneficial role of PPAR gamma agonists as anticancer therapy. An increased incidence of some cancers has been associated with PPAR agonists in rodent carcinogenicity studies. It is difficult to place preclinical findings into context given that limited data have been gathered in clinical settings. This study evaluated the association between thiazolidinediones (TZDs) and colon, bladder, liver, bile duct, gall bladder, pancreatic cancer and melanoma using the integrated rosiglitazone clinical trial database (RSG CT) (14,359 subjects) and an administrative claims database (191,223 diabetes patients).
Materials and Methods: For RSG CT data, we compared cumulative incidence rates of various neoplasms for the RSG group (N=9,542) relative to other antidiabetic agents (N=4,062). We compared the incidence rate of neoplasms in RSG CT to that of the general population using SEER 2001 and calculated standardized incidence ratios (SIRs). For claims data, we used logistic regression to calculate relative risks (RR) of cancer (overall and site-specific) for patients ever exposed to each antidiabetic category compared to non-use among a type 2 diabetes (T2D) and a general population cohort, adjusting for age, gender and cancer risk factors.
Results: In RSG CT the inidence rate per 100 patient years of exposure for any malignant, benign or unspecified neoplasm

was similar for RSG and non-RSG treatment regimens. SIRs for cancers associated with diabetes were increased in RSG treated subjects relative to general population (table 1).
In claims data, the adjusted RR comparing risk of cancer in patients ever exposed to TZD monotherapy to nonuse in T2D cohort was 0.92 and 1.76 compared to nonuse in general population (table 2).
Conclusions: The overall risk of selected cancers was higher for all antidiabetic exposure categories, including TZDs compared to the general population; consistent with the literature on cancer and diabetes.

Table 1: Standardized Incidence Rates for Selected Cancers Using RSG CT Data and SEER 2001

Site	SIR (95% C.I.)	Published data on relative risk of cancer in diabetic patients compared to the general population
Bladder	1.35 (0.49–2.93)	1.5–2.7
Breast*	0.76 (0.35–1.44)	0.8–1.4
Colorectal	0.97 (0.48–1.74)	1.0–3.0
Kidney	0.77 (0.09–2.79)	1.3–2.1
Liver	4.48 (1.46–10.46)	1.4–4.3
Lung	0.94 (0.53–1.55)	0.98
Melanoma	2.23 (0.90–4.60)	Insufficient data
Pancreas	2.12 (0.69–4.94)	1.4–4.9
Prostate**	0.74 (0.46–1.13)	0.6–1.5

* Female patients ** Male patients

Table 2: Overall Incidence of Selected* Cancers Among Patients Ever Exposed to Selected Antidiabetic Regimens Compared to Non-Use in Diabetes Patient and General Population Sample using administrative claims data (01/01/2000–12/31/2004)

	Cases	Total N	Adjusted** RR (95% CI)
TZD monotherapy			
Ever Use	153	40,797	0.92 (0.77–1.11)
Non-use (diabetes population)	625	139,448	Referent
Ever Use	153	40,797	1.76 (1.44–2.14)
Non-use (general population)	356	251,489	Referent
Sulfonylureas monotherapy			
Ever use	308	65,826	0.89 (0.77–1.04)
Non-use (diabetes population)	412	101,626	Referent
Ever use	308	65,826	1.75 (1.49–2.07)
Non-use (general population)	356	251,489	Referent
Metformin monotherapy			
Ever use	225	66,235	0.83 (0.71–0.98)
Non-use (diabetes population)	498	100,210	Referent
Ever use	225	66,235	1.67 (1.40–1.99)
Non-use (general population)	356	251,489	Referent

* Includes colon, bladder, liver, bile duct, gall bladder, pancreas and melanoma.
** Adjusted for age, gender and risk factors for cancers of interest

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0397

Achivement of treatment goals in diabetes mellitus patients – pretension and reality. The CoRiMa-Study – routine documentation of daily care

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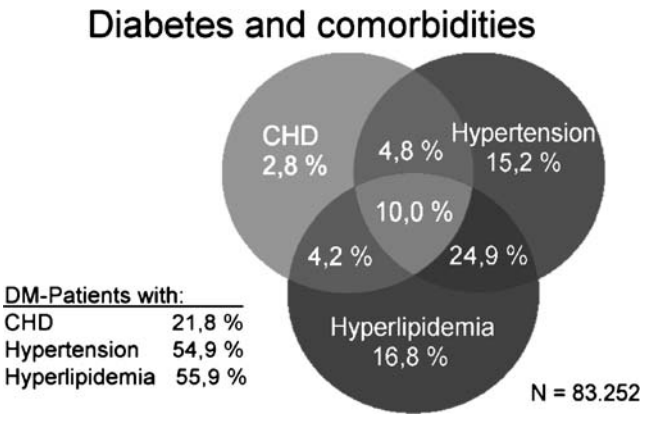
Background and Aims: As measured by prevalence and resulting expenses in public health systems, Diabetes mellitus (DM) is one of most important chronic diseases. The positive effect of a guideline conform glucose metabolism in combination with optimized blood pressure as well as cholesterol treatment is undisputed. The study objective was to investigate how well this consensus is transferred into daily practice.
Materials and Methods: Medical therapy data of patients with DM, treated between 1998 and 2005, were anonymously exported directly from patient management system in physician offices. To assess the quality of medical care, information about age, gender, blood pressure, cholesterol (Total-C, LDL-C, HDL-C) and other risk factors were evaluated. Pre-/post-analyses were carried out to compare rates of achievement of treatment goals between patients first and last office visit. The guidelines of the German Diabetes Society (DDG) were used as reference values.
Results: 83.252 patients with documented DM (ICD-10-coding and/or HbA1c≥6,5% and/or antidiabetic medication) were included. Most of them had at least one comorbidity: 54,9% hypertension, 55,9% hyperlipidemia, 21,8% confirmed coronary heart disease (CHD), and approximately 10,0% had all 3 comorbidities **(Fig.)**. The rates of treatment goal achievement were as follows: LDL-C 28,6%, Blood pressure 38,7%, HbA1c 35,7%. Considering the LDL-C distribution of patients with DM and CHD, about 66,2% showed LDL-C≥100 mg/dl at the final visit.
Conclusion: Compared with DDG guidelines, remarkable deficiencies in medical care of patients with DM were detected. Possible causes could be underestimated cardiovascular risks, deficiency in patient's motivation as well as budget limits.



0398

Normal life expectancy in non-smoking, well-controlled type 2 diabetes patients

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Background and Aims: Studies of the 20th century reported increased mortality in type 2 diabetes (T2DM). Since there have been major changes in treatment regimes with more stringent goals for metabolic control and cardiovascular risk management, improvement of life expectancy in T2DM could be expected. In this study, we aimed to assess whether life expectancy of T2DM-patients has indeed changed.

Materials and Methods: In 2001, 973 primary care T2DM-patients, participating in a shared-care project, were included. This group (mean age 66 yrs) was well-controlled (mean HbA1c 7.0%) and had a relatively short diabetes duration (median 4.2 yrs). Vital status was assessed at January 2005. Life expectancy was assessed by transforming survival time to standardised survival time (SST) allowing adjustment for the baseline mortality rate of the general population.

Results: During a median follow-up of 3.2 years, 86 patients died (44 from cardiovascular disease). There was no reduction of median life expectancy in T2DM, compared to the general population (SST=0.96 vs. 1.0). However, mortality was increased in the smoking T2DM-group compared to the general population at SST=0.25 (0.196 vs. 0.070, $p<0.01$) and SST=0.5 (0.335 vs. 0.169, $p<0.02$). Smoking (HR 2.0), peripheral vascular disease (PVD) (HR 2.2), and albuminuria (HR 1.2) were significant predictors ($p<0.01$) for all cause mortality in multivariate analysis; HbA1c, blood pressure, lipids, and a history of ischemic heart disease, were not.

Conclusion: This study shows a normal life expectancy in a non-smoking and well-supported T2DM population. Smoking, presence of PVD, and to a lesser degree albuminuria, however, reduce life expectancy. These results show that a normal life expectancy is achievable in T2DM-patients.

This study was supported by a grant from the Dutch Diabetes Research Foundation (project 2000.00.06) and the Medical Research Foundation Zwolle

0399

Treatment and diagnosis of hyperlipidaemia in diabetes mellitus – awareness of an essential risk factor for cardiovascular disease – results from the German CoRiMa-Study

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Background and Aims: Patients with diabetes mellitus (DM) have a high risk for cardiovascular disease, and they often have several additional risk factors. Identification of these risk factors is therefore important to develop adequate therapies to reduce morbidity and mortality. Thus, the present study focused on diagnostic accuracy and treatment of hyperlipidemia. Results according to total (TC) and LDL cholesterol (LDL-C) are compared to international guidelines.

Materials and Methods: In selected (general practitioners and internal medicine) physician offices in Germany, all patients with a diagnosis of DM seen between 1998 and 2005 were identified. The routine documentation was exported from the physician's patient management system into a project database. DM patients were selected based on 2 criteria: ICD-10 coding of DM and plausibility check for DM (i.e. use of anti-diabetic medication and/or $HbA_{1c} \geq$ 6,5%). The identification of hyperlipidemia was assessed using 3 criteria: ICD-10 coding, TC levels ≥190 mg/dl and/or use of lipid-lowering medication. To evaluate the status of care, treatment, age, gender, serum lipids levels, and information regarding additional risk factors were analyzed. The last office visits were used for comparisons with

treatment goals stated in current guidelines which were used as reference values for treatment evaluation.

Results: Overall, 83.252 patients with DM were identified: 88,7% by ICD-10 coding, 9,1% by antidiabetic medication use and 2,2% based on HbA_{1c} values≥6,5% at the last office visit without any documented antidiabetic medication. Adding plausibility checks, a total of 46.565 patients (55,9%) with hyperlipidemia were identified, of whom only 22.931 had ICD-10 coding of hyperlipidemia. About 29,0% of patients without ICD-10 coding for hyperlipidemia were detected by use of lipid-lowering medication, and 70,1% by TC ≥190 mg/dl without lipid-lowering medication. Patients **with (P1)** and **without (P2)** documented hyperlipidemia (ICD-10), the following percentages achieved the goals of treatment: **P1:** TC 29,6%; LDL-C 25,7%; blood pressure 38,0%; HbA1c 38,7%; **P2:** TC 12,7%; LDL-C 17,2%; blood pressure 36,7%; HbA1c 33,7%. In addition to hyperlipidemia, hypertension and coronary heart disease were present in 54,9% and 21,8%, respectively. At the last visit, 74,3% of patients in **P1** and 82,9% in **P2** had LDL-C≥100 mg/dl.

Conclusion: Compared with current guidelines, the majority of patients with DM does not achieve treatment goals for other relevant modifiable risk factors. Comparing ICD-10 coding with hyperlipidemia diagnosed by plausibility checks, there is a significant prevalence of undetected hyperlipidemia in patients with DM. This lack of awareness of hyperlipidemia may be the cause for the high rates of LDL-C≥100 mg/dl.

0400

Glycaemic control in type 2 patients starting insulin therapy – a retrospective study

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Background and Aims: When using the traditional stepwise approach to treating type 2 diabetes, most patients progress from lifestyle interventions (e.g., diet and exercise) to oral anti-diabetic drugs (OADs), followed by insulin, with or without OADs. There is increasing recognition that this inherently reactive strategy to progressive treatment failure subjects patients to suboptimal glycaemic control. This study aimed to investigate at what stage of glycaemic control patients in real-life clinical practice began insulin therapy, and how the routine initiation of insulin affected their glycaemic control.

Materials and Methods: An anonymised computerised UK primary care database (DIN-LINK) was used to identify type 2 diabetes patients who were issued their first prescription of insulin between March 2002 and April 2005. Only patients who had a previous prescription for oral anti-diabetic therapy were included in the analysis to exclude type 1 patients and those who had moved from another district.

Results: The database identified 2,040 type 2 patients who were issued their first prescription of insulin, of whom 1,790 had an HbA1c reading before the prescription was issued. The mean age was 63.8 years (SD=12.8), 45% of patients were female with a mean HbA1c of 9.8% (SD=2.0%) before insulin initiation. After 6 months 774 patients had a second HbA1c recorded, mean HbA1c reduced by 1.4% ($p<0.001$). This decrease was maintained at 1 year where 475 patients had a recorded HbA1c.

Summary of absolute HbA1c (%) for all newly initiated patients

Months from 1st insulin prescription	0	6	9	12	15	18	21	24
Number of patients	1790	774	599	475	394	307	226	199
Mean (%HbA1c)	9.75	8.31	8.19	8.22	8.29	8.36	8.27	8.26
Median (%HbA1c)	9.40	8.10	7.90	8.00	7.90	8.20	8.10	8.20
Standard deviation	1.97	1.42	1.45	1.41	1.55	1.51	1.49	1.42

Conclusion: Commencing insulin therapy in type 2 diabetes significantly improves glycaemic control; however HbA1c in patients starting insulin therapy is much higher than current recommended targets such as those set by NICE. These data indicate that more aggressive efforts are needed to initiate insulin at an earlier time point in the progression of type 2 diabetes.

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0401

Hypoglycaemia and self-monitoring blood glucose are predictive factors for achieving glycaemic control in people with diabetes

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Background and Aims: To identify predictive factors for achieving a target of HbA_{1c} <7% in patients with type 1 (T1DM) and type 2 diabetes (T2DM) world wide, a logistic regression analysis was performed using initial data gathered in the International Diabetes Management Practices Study (IDMPS) in 11 countries (Eastern Europe, Asia, Latin America).
Materials and Methods: 1327 people with T1DM and 7543 people with T2DM were recruited; 798 T1DM patients and 3537 T2DM patients were available for the logistic regression analysis.
Results: In the overall population, the mean HbA_{1c} was 8.2±1.96% in T1DM patients (27% had an HbA_{1c} <7%) and was 7.7±1.75% in T2DM patients (38% had an HbA_{1c} <7%). It was found that prior hypoglycemia or a fear of hypoglycemia largely decreases the chance that people with diabetes will achieve the HbA_{1c} target: people with T1DM have a 12-times greater chance (95% confidence interval [CI]: 5.106; 28.070) of achieving the target if they did not experience, or are not afraid of, hypoglycemia and the chance is 17-times greater (95% CI: 6.118; 45.769) for people with T2DM. It was also found that self monitoring of blood glucose increased the likelihood of achieving the target of HbA_{1c} in both T1DM patients (2.2-times greater, 95% CI: 0.265; 0.770) and T2DM patients (1.3-times greater, 95% CI: 0.648; 0.886). The only other factors found to correlate with achieving the HbA_{1c} target were whether patients had a normal body mass index (20–25 kg/m^2), self-adjust their insulin dose, not having a macrovascular complication and had a shorter duration of disease (1–5 years).
Conclusion: Thus, the factors most strongly associated with achieving the recommended target of HbA_{1c} <7% in people with either T1DM or T2DM were whether people previously experienced, or were afraid of, hypoglycemia and self-monitoring of blood glucose.
Supported by Sanofi-Aventis

0402

Effect of orlistat on weight regain and development of type 2 diabetes following a very-low-energy diet in abdominally obese patients. A three-year-randomised placebo controlled study

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Objective: To investigate the efficacy of orlistat on the maintenance of weight loss over three years following a major weight loss induced by very low energy diet (VLED) in abdominally obese patients with metabolic risk factors including impaired glucose tolerance.
Study design: Initially, weight loss was induced by an eight-week VLED (600-800 kcal/day) in 383 obese patients with a mean BMI of 37.5 kg/m^2 (range 30.0-45.2 kg/m^2). Those who lost>5% of their body weight (309 of 383 patients) were then randomized to receive orlistat 120 mg t. i.d. or matching placebo capsules and life style counselling for three years. Primary endpoints were the maintenance of>5% weight loss and the absolute weight loss after three years. Additionally, we analysed differences in the development of type 2 diabetes between orlistat and placebo.
Results: The VLED induced a mean weight loss of 14.4±2.0 kg among the subsequently randomized patients. The mean weight gain after the three years was lower with orlistat than with placebo (4.6±8.6 kg vs. 7.0±7.1 kg; P<0.02). The number of participants who achieved>5% weight loss favoured also orlistat (67% vs. 56%, P=0.037). Waist circumference was significantly more reduced in the orlistat group (P<0.05) but no other differences in the risk factors were observed between the two groups. The incidences of new cases of type 2 diabetes were significantly reduced in the orlistat group (5.2%) versus placebo (10.9%) (P=0.041).
Conclusion: The addition of orlistat to lifestyle intervention improved the maintenance of weight loss after VLED for up to three years in abdominally obese subjects. Even a modest extra weight loss induced by orlistat was associated with a reduced occurrence of type 2 diabetes.
Supported by Roche Scandinavia

0403

The twenty-year outcomes – the 1984 insulin-treated diabetes cohort enrolled on the Canterbury diabetes registry

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Background and Aims: Despite improvements in the prognosis of individuals with diabetes, mortality rates in patients with Diabetes Mellitus are still two- to five-fold higher than in the general population despite many recent improvements in care. Most often this premature mortality is attributed to the long-term cardiovascular and renal complications of diabetes. Countless health initiatives have been put in place to reduce the heavy burden of disease in these patients by better recognition, earlier treatment and reduction of long-term complications. Many studies have assessed the success of these measures determining the accurate measurement of morbidity and mortality rates in

diabetes. However these rates vary substantially from country to country and require long follow-up periods. If diabetes mortality, and changes in diabetes mortality, are to be studied accurately in New Zealand, then baseline mortality rates specific to the New Zealand population must be established.

Aims: To establish cause-specific death rates, by age and sex in insulin-treated diabetic individuals living in Canterbury, NZ.

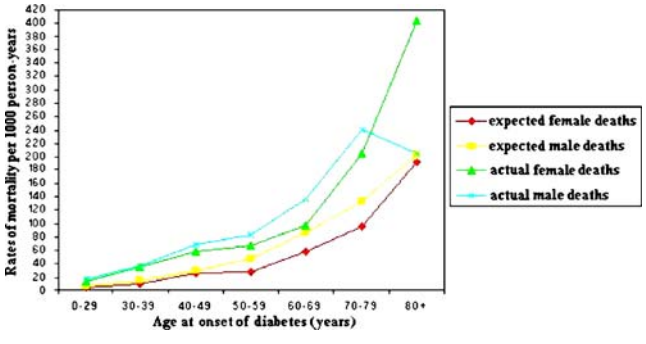
Materials and Methods: Insulin-treated diabetic subjects (n=995) on the Canterbury Diabetes Registry were followed up over 20-years and vital status determined. Following notification of deaths during this period, age- and sex-specific mortality rates, standardised mortality rates and standardised mortality difference were calculated.

Results: Six subjects had left NZ leaving 989 diabetic subjects who contributed 13,706 person–years of follow-up. During follow-up 525 deaths occurred in subjects aged 17.3 to 96.7, 261 in females and 264 in males. At all ages mortality rates were considerably higher than expected mortality. The SMRs were higher for females than males at all ages, being 2.48 (95% CI 2.18 to 2.78) for females and 2.17 (95% CI 1.91 to 2.43) for males overall. Relative mortalities were increased for cardiovascular, renal, respiratory disease and malignancy with cardiovascular disease accounting for the single greatest cause of excess death at all ages.

A comparison of actual and expected mortality rates, by age at onset of diabetes, for the cohort per 1000 person–years is shown in Fig.1. All-cause mortality rates increased with age, and, were higher in diabetic men than diabetic women at all ages except ≥80 years. However numbers surviving to this age were very small (n=2 men and 4 women). Actual mortality rates were also higher at all ages in diabetic women than the expected deaths in non-diabetic men.

Conclusion: This is the longest follow-up study carried out in New Zealand and shows that mortality rates for diabetic individuals remain high relative to the general population. To reduce these death rates attention must be paid to the early detection and treatment of cardiovascular disease and associated risk factors.

Figure 1: Expected and actual mortality rates per 1000 person–years



Supported by Canterbury Medical Research Foundation

0404

Unsatisfactory risk factor control in type 1 diabetes 1997–2004: results from the Swedish National Diabetes Register (NDR)

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Background and Aim: In order to reduce micro- and macrovascular complications in type 1 diabetes (T1D), it is vital to improve the glycaemic control as well as other risk factors. In 2004 more than 100.000 type 1 and type 2 diabetic patients were reported to the NDR, which is one of the largest diabetes registries in the World. The aim of this survey was to describe the clinical characteristics, glycaemic and risk factor control in cross-sectional and longitudinal cohorts of T1D patients from 1997 to 2004.

Material and Methods: NDR was initiated as a tool of quality assurance in diabetes care in hospital outpatient clinics and primary care centres. Patients were reported annually to the NDR by physicians and nurses, via paper forms, computer software or via the Internet. Clinical characteristics, HbA1c, treatment and risk factors were registered. The definition of T1D was age of debut <30 years in combination with insulin treatment only. In 2004, 13.612 T1D patients (age≥18 years) were reported. This represents about half of the Swedish T1D population.

Results: In 2004 the mean age of the T1D patients was 41.6±SD13.5 years, the mean duration of diabetes was 26.1±14.1 years and 46% of the patients were female. The mean HbA1c decreased from 8.2±1.3% in 1997 to 8.0±1.2% in 2004 ($p<0.001$). The proportion of patients reaching the national goal for HbA1c (<7.3%, DCCT standardized) increased form 24.8 to 29.6% ($p<0.001$). A slow but significant improvement in blood pressure levels was seen, but only 61.3% reached the blood pressure goal (≤130/80). There was a slight increase in BMI from 24.9±3.5 kg/m^2 in 1997 to 25.3±3.9 kg/m^2. The use of antihypertensive and lipid lowering agents increased, whereas smoking habits were virtually unchanged. Logistic regression analyses showed that successful glycaemic control (HbA1c <7.3% both in 1997 and in 2004) was predicted by low BMI in 1997, weight loss from 1997 to 2004, non-smoking and absence of microalbuminuria.

Conclusion: In this large cohort of Swedish T1D patients treated in everyday clinical practice, there was a slow improvement in glycaemic and risk factor control from 1997 to 2004, but the gaps between clinical results and

treatment goals were still big. Only 17% of the T1D patients reached the treatment goals for HbA1c, blood pressure and cholesterol. Risk factor control in T1D is still unsatisfactory and it is crucial that new measures are taken in order to improve diabetes care.

PS 16

Acute and chronic complications in diabetes

0405

Diabetic ketoacidosis in Denmark. Incidence and mortality estimated from public health registries

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Background and Aims: Previous studies have reported an increasing incidense of diabetic ketoacidosis (DKA) and a decreasing mortality from DKA in Denmark.
The aims of the present study were to estimate incidence of and mortality from DKA in Denmark and to characterise deaths associated with DKA using data from national health registries.
Materials and Methods: Hospital admissions in the period 1996–2002 with a discharge diagnosis of DKA were identified from the Danish National Patient Registry (NPR). Deaths associated with DKA in the period 1996–2000 were identified from the Danish Cause of Death Registry (CDR).
Results: A total of 4807 admissions and 137 deaths with a diagnosis of DKA were identified. Annual incidence of DKA in the general population was estimated to 12,9 per 100.000. The annual incidence was higher in males than in females (14.4 vs. 11.4 per 100.000, $p<0.0001$). 12% of all patients were classified as Type 2 DM, and this percentage increased exponentially with age>50 years.
The mortality was 4%. In patients>70 years the mortality was higher than in younger patients (15% vs. 2%, $p<0.0001$). One or more additional somatic diagnoses were stated on 77% of the death certificates, most often of a cardiovascular or infectious disease (47% and 30% respectively)
Conclusion: DKA is still a frequent complication to diabetes, but the number of admissions related to DKA has remained the same during the past 25 years despite an increasing prevalence of diabetes, however the incidence seems to have decreased in younger patients with Type 1 DM. Older patients with DKA differed from younger patients in having a higher mortality and a larger proportion of patients classified as Type 2 DM.

0406

Poor metabolic control in diabetic patients: a risk factor of shortsightedness

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Background and Aims: As a consequence of an increasing prevalence of shortsightedness (myopia) in parts of the world that has adopted western dietary patterns it has been hypothesized that hyperglycaemia and hyperinsulinaemia induce myopia. The purpose of this study was to evaluate the relation between glycosylated haemoglobin (HbA_{1c}), insulin dosage and myopia in diabetic patients.
Materials and Methods: A total of 383 type 1 diabetic patients aged 16–26 years (mean age 22.0 (SD 2.9)) attending the eye clinic at Steno Diabetes Center, Copenhagen, in 1995–97, were included in a retrospective cohort study. The following data were collected from the medical records from baseline to 2005: age at diabetes onset, age at baseline, sex, weight, HbA_{1c}, insulin dosage, refractive error (automated refraction in cycloplegia), visual acuity and diabetes related complications.
Results: The prevalence of myopia (≤ -0.5 D, spherical equivalent) was 53.3% (95%CI (confidence interval)±5.29) at baseline and 65.6% (95%CI±5.88) at the last eye examination (mean follow-up 7.1 years (SD 2.2). In a logistic regression analysis, duration of diabetes (OR (Odds ratio) 0.918 (95% CI 0.873; 0.966), p=0.001), refraction at baseline (OR 0.858 (95% CI 0.742; 0.992), p=0.038) and HbA_{1c} (OR 1.270 (95% CI 1.058; 1.526), p=0.011) were related to myopia, whereas insulin dosage was not (OR 1.833 (95% CI 0.595; 5.647), p=0.291).
Conclusion: This study suggests a relation between development and progression of myopia and poor metabolic control in diabetic patients. In addition, myopia was more frequent in the present study population than in non-diabetics.
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0407

Increased risk of common infections in hospitalised patients with type 2 diabetes mellitus

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Background and Aims: Many clinicians believe that people with diabetes are more susceptible to infections. Although certain rare infections are more common, risks of acquiring an infectious disease, as well as infection-related mortality, are not well studied for diabetic patients.
Materials and Methods: A retrospective cohort study was conducted, comparing all patients with any common infection hospitalized in our clinic during a decade, (1990–2000), to matched patients hospitalized for any reason other than infection. The risk ratios of having an infectious disease and of in-hospital death attributable to infectious disease between those with and without diabetes were calculated, using multiple regression analysis models. The results were adjusted for possible confounding factors (age, sex and history of cardiovascular disease).
Results: The cohort consisted of 2583 patients hospitalized for any common infection (491 (19.01%) with diabetes and 2092 without) and 1408, matched for age and sex, patients hospitalized for any reason other than infection (199 (14.13%) with diabetes and 1209 without). The risk of acquiring an infectious disease was 28% higher for diabetic patients compared to those without diabetes (OR=1.28 95% CI 1.06–1.55 p=0.010). There were 323 (12.50%) deaths among the 2583 patients hospitalized with infection and 100 (7.10%) deaths among the 1408 hospitalized for any other reason. Overall, the risk of death was about two times higher for patients hospitalized for any common infection (OR=2.36 95% CI 1.81–3.07 p<0.001). Among patients hospitalized for any common infection, there were 68 (13.85%) deaths among the 491 diabetic patients and 255 (12.19%) deaths among the 2092 non-diabetic patients. The risk of death attributable to infectious disease did not differ between hospitalized diabetic and non-diabetic patients (OR=1.12 95% CI 0.81–1.53 p=0.497).
Conclusion: The risk of acquiring an infectious disease is higher among diabetic patients compared to their non-diabetic counterparts. On the other hand, infection-related mortality did not seem to differ between hospitalized diabetic and non-diabetic patients, although, overall, the risk of death attributable to infectious disease was quite high.

0408

Care and outcomes in 35,723 adults with diabetes mellitus in Germany 2005

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Background and Aims: The German Diabetes Association provides a certificate of quality for hospitals (HO) and specialised private practices (PR). Candidates have to provide information on the staff, patient education and documentation of patient data. 27 items have to be submitted on>50 patients with type 1 diabetes (DM1) and 200 patients with type 2 diabetes (DM2). The aim of the analysis presented was to describe the quality of care of adult persons with diabetes mellitus in a large sample in Germany.
Materials and Methods: In 2005 119 institutions (27 PR, 92 HO) applied for the certificate. 35,723 patients (5,891 DM1, 29,693 DM2, 100 pancreas diabetes) out of 26 PR (1,664 DM1, age 43.7 y, years sime since diagnosis 15.7, BMI 25.9 kg/m^2, 7,779 DM2, 66.9 y, 9.9 y, 30 kg/m^2) and 92 HO (4,227 DM1, age 43.9 y, time since diagnosis 15.8 years, BMI 25.1 kg/m^2, 21,914 DM2, 64.3 y, 9.9 y, 31.1 kg/m^2). HbA1c values were DCCT adjusted. The data from PR represent a cross section of care and outcome, in contrast to the data from hospitals which describe the quality of care at the time of admission.
Results:
Diabetologists' practices:
DM1 (n=1664) age 43,7J; years since diagnosis 15.7; BMI 25.9 kg/m^2; HbA1c 7.22% (HbA1c <7%: 48.5%; HbA1c>7%: 51.5%); RR 124/74 (RR sys.>140 or RR dia. >90: 16.4%); severe hypoglycaemia 0.06/pat/y; severe ketoacidosis 0/pat/y.
IDDM 2: (n=3664) age 65.3J; years since diagnosis 12.,6; BMI 30.6 kg/m^2; HbA1c 6.96% (HbA1c <7%: 59.8%; HbA1c>7%: 40.2%); RR 135/77 mm hg (RR sys.>140 or RR dia.>90: 30.4%); severe hypoglycaemia 0.01/pat/y; coma with hospitalisation 0/pat/y
NIDDM 2: (n=4115) age 65.3J; years since diagnosis 6.8; BMI 31.6 kg/m^2; HbA1c 6.65% (HbA1c <7%: 69.7%; HbA1c>7%: 30.3%); RR 135/79 (RR sys.>140 or RR dia. >90: 31.7%); severe hypoglycaemia 0/pat/y; coma with hospitalisation 0/pat/y.
Hospitals:
DM1 (n=4227) age 43.9 y; years since diagnosis 15.8; BMI 25.1 kg/m^2; HbA1c 8.2% (HbA1c <7%: 33,4%; HbA1c>7%: 66,6%); RR126/76 mm hg (RR sys.>140 or RR dia.>90: 15.9%); severe hypoglycaemia 0.3; severe ketoacidosis 0.11/pat/y
IDDM2 (n=7523) age 66.9 y; years since diagnosis 12.0; BMI 30.3 kg/m^2; HbA1c 7.97% (HbA1c <7%: 36.6%;

HbA1c>7%: 63.4%); RR137/78 mm hg (RR sys.>140 or RR dia.>90: 31.5%); severe hypoglycaemia 0.08/pat/y; coma with hospitalisation 0.02/pat/y.
NIDDM2 (n=14391) age 66.9 y; years since diagnosis 6.0; BMI 29.5 kg/m^2; HbA1c 6.98% (HbA1c <7%: 63.0%; HbA1c>7%: 37.0%); RR136/79 mm hg(RR sys.>140 or RR dia.>90: 30.1%); severe hypoglycaemia 0.03/pat/y; coma with hospitalisation 0.01/pat/y.
Conclusion: In comparison to patients on continuous care in specialised private practice patients on admission to hospitals were older, diabetes duration was longer, BMI and HbA1c were higher and they had more acute complications. 50% of the patients with type 1 diabetes taken care of in practices of diabetologists have very good HbA1c-value <7%, respectively; 60% of patients with type 2 diabetes on insulin and 70% of patients with type 2 diabetes without insulin treatment. Acute complications occur very rarely – altogether the data represent a very satisfactory quality of care. In patients on admission to hospitals Hba1c above 7% is present in 67% of patients with DM1, 63% of patients with DM2 and insulin therapy and 37% of patients with DM2 without insulin therapy at admission to hospital – not unexpected since the patients were admitted to the hospitals due to problems of care. The general quality of documented blood pressure was excellent.

0409

Frequent microangiopathy in fulminant type 1 diabetes – a nationwide, 5-year-study

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Background and Aims: Fulminant type 1 diabetes (FT1D) is a novel subtype of type 1 diabetes, which is characterized by a markedly acute onset of diabetes and an absence of islet-related autoantibodies. The aim of the present study was to assess the development of diabetic microangiopathy in FT1D.
Materials and Methods: Forty-one patients with FT1D (21 males and 20 females, age at onset; 38.7±15.1 yrs) and age- and sex-matched 76 patients with type 1A diabetes (T1AD) had been followed for 5 years in a nationwide survey in Japan. The following data were recorded every 12 months after the onset of diabetes: Seven point (preprandial and 120-min postprandial for each meal and bedtime) blood glucose concentrations, HbA_{1c} level, urinary albumin excretion, fasting or postprandial serum C-peptide level, body weight, systolic and diastolic blood pressure, daily dosages of insulin, frequency of severe hypoglycaemic episodes, presence or absence of Achilles' tendon reflex, and the result of fundoscopic examination by ophthalmologist.
Results: Five-year cumulative incidence of microangiopathy was 24.4% in FT1D and 2.6% in T1AD. In longitudinal studies using Kaplan-Meier method, the incidence of each microangiopathy was significantly higher in FTID than in T1AD; retinopathy was 9.8% vs. 1.3% (p=0.033), nephropathy 12.2% vs. 2.6% (p=0.015), and neuropathy 12.2% vs. 1.3% (p=0.010), respectively. Mean HbA_{1c} levels were similar in FT1D and T1AD group during the follow-up periods (7.4±1.3% vs. 7.7±1.8%, NS). However, mean insulin dosages, mean M-value, and the frequency of severe hypoglycaemic episodes were significantly higher in FT1D than in T1AD (0.76±0.22 vs. 0.60±0.21 U/kg, p=0.0001, 29.2±13.5 vs. 23.0±15.3, p=0.047, and 0.30±1.04 vs. 0.01±0.06 episodes/yr/person, p=0.018, respectively). Blood pressure and body weight did not differ between the two groups. Significantly lower levels of mean postprandial C-peptide were observed in FT1D (0.26±0.12 vs. 0.71±0.44 ng/ml, p=0.0007).
Conclusion: These data suggest that FT1D is a high-risk subgroup of diabetic microangiopathy, probably through brittleness in blood glucose levels due to the lack of endogenous insulin secretion from onset of diabetes.

0410

Prevalence of diagnosed diabetic retinopathy and its severity in four European countries

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Background: Type 1 and type 2 diabetes mellitus are associated with a broad spectrum of microvascular complications, including DR. An understanding of the prevalence of DR and its severity is essential to an appreciation of the full burden of diabetes. The characteristics of DR are not well defined in the literature.
The primary objectives of this study were:
• To estimate the prevalence of diagnosed DR in patients with type 1 and type 2 diabetes mellitus; and
• To characterize and estimate the prevalence of the severity stages of DR.
Methods: This study was a cross-sectional survey of physicians who practice within the primary care (GP) setting in France, Italy, Spain and the United Kingdom (UK); additionally diabetes specialists were recruited in Italy and Spain. Information on the prevalence of diagnosed DR was obtained from existing physician records. Clinical details were collected from a random sample of DR patients using medical records.
Results: Forty-one clinics (mean) per country were included in the study (range: 34–49). The estimated prevalence of diagnosed DR amongst GP patient populations ranged from 9.6% in Spain to 19.6% in the UK. In Italy and Spain, prevalence among Diabetic Specialist (DS) patient populations was 20.2% and 32.7%, respectively. In Spain, this was three-fold higher than prevalence in primary care, while no substantial difference was noted in Italy.

Prevalence of Diagnosed DR per 100 Diabetic Patients (95% CI) per Country

France*	Italy	Spain	UK*
11.4	**GP:** 18.3	**GP:** 9.6	19.6
(8.8–13.9)	(6.9–29.8)	(5.9–13.3)	(16.0–23.1)
	DS: 20.2	**DS:** 32.7	
	(14.01–26.4)	(18.2–47.3)	

*Data collected only from GPs

Charts were abstracted for 752 patients with DR. Of these 53.9% were males and 66.9% had type 2 diabetes. Mean age (+SD) was 64.2+12.8 years; HbA1c was 7.75+1.4% (Mean+SD). DR diagnosed as proliferative ranged from 19.7% in France to 31.5% in the UK. Consistently across countries, mild Non-Proliferative DR (NPDR) was the most common severity level of NPDR.

Distribution of Severity Levels per 100 DR Patients by Country

DR Type	France	Italy	Spain	UK
NPDR				
Mild	40.9%	39.2%	43.2%	42.0%
Moderate	27.3%	27.8%	19.4%	16.0%
Severe	1.5%	4.2%	2.6%	3.3%
Proliferative DR	19.7%	21.7%	29.1%	31.5%

Hypertension (70.1%), dyslipidaemia (61.0%) and neuropathy (53.2%) were the most common co-morbidities. Nephropathy was present in 38.5% of patients with mild NPDR and in 64.4% of patients with proliferative DR.
Conclusion: In this multi-national study, country-specific prevalence estimates of diagnosed DR may reflect respective clinical practice in the management of diabetes and its complications. Consistently across countries, the majority of diagnosed NPDR is of mild to moderate severity.
Supported by Eli Lilly and Company

0411

Decreasing prevalence of diabetic retinopathy in 1828 outpatients from the university diabetic clinic Jena in 2005 and 2006

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Background and Aims: The incidence of diabetic retinopathy (DR) has decreased due to improvements in diabetes care (Hovind, Diabetes care 2000). However, there is little knowledge concerning the incidence of retinopathy in Germany. The objectives of this study were to estimate the incidence and risk factors for DR using routinely collected data from a clinical information system at the University Jena (Germany) for type 1 and 2 diabetes.
Materials and Methods: We studied results of fundoscopy and clinical features of 1828 patients (943 male and 890 female) with diabetes mellitus (DM1 21.7%, age 46, years since diagnosis 19; DM2 78.2%, age 63.4, years since diagnosis 13.5) who presented in 2005 and 2006 at the outpatient clinic. Data were obtained from the electronic system EMIL®. The mean age of the participants was 60 (±15) yr with a mean duration of diabetes of 14 (±10) yr. Ophthalmological examinations of 1229 patients (67.2%) were available. The mean follow-up was 5 (±5) (range 0–20) yr.
Results: Diabetic retinopathy was found in 30.2% of patients (DM1 37.1%; DM2 27.8%): non-proliferative retinopathy (NPDR) 234 patients (19%), proliferative (PDR) 11 (9,0%), blindness 8 (0,7%), clinically significant macular edeme (CSME) 29 (2.4%). Regarding the type of diabetes:
DM1: no RD 62.8%, NPDR 22.3%, PDR 11.8%, blindness 0.6%, CSME 3.1%. The incidence of RD depending on duration of diabetes was: 0≤5 years 0%; 5≤10 7.1%; 10≤15 17.7%; 15≤20 33.8%; 20≤25 52.7%; 25≤30 60.9%;≥30 86.6%.
DM2: no RD 72.2%, NPDR 17.9%, PDR 8.1%, blindness 0.9%, CSME 2.1%. The incidence of RD depending on duration of diabetes was: 0≤5 years 5.4%; 5≤10 15%;

10≤15 17.5%; 15≤20 40.8%; 20≤25 59.6%; 25≤30 61.7%;≥30 54.4%.

No patient with DM1 and duration of diabetes less than 5 yr had retinopathy, but 3,0% of patients with DM2 exhibited NPDR and 1.8% had PDR. Among patients with DM1 after 5–10 years of the disease no patient had PDR, but NPDR was present in 7.1%. Among patients with DM1 PDR was first detected by 10<15 yr duration and was present in 3.7%. 59% of patients with duration>30 yr had no retinopathy (DM1 13.3%; DM2 45.7%). In this group the PDR was present in 33.3% of DM1 and 17.4% of DM2. The major part of patients with duration>30 years had the NPDR. Of those patients with RD, 95.4% were insulin-treated, 4.5% with oral antiglycemic agents and 82.3% with antihypertensive medications. Of the patients without RD 83%, 19% and 74.7%, respectively. The degree of DR was found to be related to the duration of diabetes, glycaemic control (HbA1c), systolic blood pressure and serum creatinine. In DM1, BMI, urinary albumin secretion and creatinine clearance were significantly higher among patients with PDR comparing to patients without RD.

Conclusion: Our study confirms the decreasing incidence of diabetic retinopathy. We provide contemporary data on the prevalence and risk factors for diabetic retinopathy among patients from outpatient diabetic clinic in Germany. Compared to a study from 1989 (Weimar, Germany) which used the register of the centralized diabetes care system in the former German Democratic Republic, we observed a decrease of diabetic retinopathy in patients with DM1 (52.5% vs. 37.3%) and in patients with insulin-treated DM2 (50.3% vs. 31.5%). Diabetic retinopathy was found in 30% of diabetic patients, a lower rate than in Wisconsin Epidemiologic Study.

0412

Prevalence of diagnosed Diabetic Peripheral Neuropathy (DPN) and its symptoms in four European countries

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There is limited consensus on how best to diagnose DPN, yet patients are diagnosed and the magnitude of severity and clinical details are not well understood. A cross-sectional survey was administered to 125, general practitioners (GP) in France, Italy, Spain, and the United Kingdom (UK) to estimate the prevalence of diagnosed DPN within practices. Prevalence estimates ranged from 9.5% in Spain to 14.1% in the UK. Additional surveys of Diabetic Specialists in Italy and Spain found a prevalence that was two-fold higher in Italy (26.8%) compared with GPs, while a smaller difference was noted in Spain (15.0%).

Prevalence of Diagnosed DPN per 100 Patients (95% CI) from GP Surveys

France	Italy	Spain	UK
10.7 (7.6–13.8)	13.9 (9.0–18.8)	9.5 (6.9–12.1)	14.1 (10.6–17.7)

Medical chart abstraction was conducted to collect data from a random sample of 913 DPN patients within the clinics. Of these, 55.0% were males and 78.5% had type 2 diabetes. Age was 64.5+12.5 years and HbA1c was 7.67+ 1.4% (Mean +SD). Approximately 27% of patients were diagnosed without a documented neurological exam. Hypertension (72.7%) and dyslipidemia (64.8%) were the most common co-morbidities. DPN diagnosis was mostly based on symptoms consistent with DPN. The most common DPN symptoms were numbness and prickling across all countries. Overall, approximately 24% of patients had no record of pain symptoms, while 26% had aching, burning and stabbing pain symptoms. Over 22% of patients were treated with anticonvulsants and 12.2% with tricyclic antidepressants.

Frequency of DPN-Related Symptoms Recorded in Medical Charts

DPN Symptoms	France	Italy	Spain	UK
Aching pain	32.3%	35.4%	37.1%	48.8%
Burning pain	44.8%	56.9%	47.4%	60.8%
Prickling	81.0%	68.0%	73.7%	49.8%
Numbness	66.4%	69.7%	77.7%	63.6%
Stabbing pain	24.1%	27.6%	31.4%	44.5%
Tenderness	50.4%	43.8%	48.0%	43.5%

Country-specific prevalence estimates of diagnosed DPN may reflect clinical management of diabetes and DPN. Consistently across countries, over a quarter of patients had a record of pain symptoms associated with DPN.

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0413

Development of complications and costs after diagnosis of type 2 diabetes: results from the ROSSO study

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Background and Aims: The German multi-centre, retrospective cohort study ROSSO observed a representative cohort of 3142 newly diagnosed type 2 diabetes patients with a public health insurance plan from randomly contacted 192 GP practices. The patients were followed over a mean of 6.5 years. The ROSSO cohort was used to analyse the development of complications and the total treatment costs.
Materials and Methods: The data of all patients of a practice with a type 2 diabetes diagnosis between 1995–1999 were taken directly from the physician's files. Monitoring assured the completeness and quality of the data collection. Based on these data, costs were attributed using the doctor's tariff, hospital DRGs and medication price lists for Germany.
Results: At diagnosis of type 2 diabetes previous diagnosis of myocardial infarction (MI) was present in 4% of the patients, stroke 3.2%, congestive heart failure (CHF) 15.3%, polyneuropathy (PNP) 5.0% and depression 7.3%. During follow up over 7 years the complication rate rose linearly to 7.3% for MI, 8.6% for stroke, 33.4% for CHF, 19.7% for PNP and 15.9% for depression.

Other diabetic endpoints as hemodialysis (1.4%) amputation (1.2%) and blindness (0.6%) rose but stayed lower. Total cost of diabetes care was 1288 € for the first treatment year with diagnosed diabetes and amounted to 3845 € in the year seven. Even in the first years after diagnosis 58% of the cost was spent for the treatment of complications and this proportion increased to 83% in the seventh year. Average costs per patient and year were 3210 €, while 6% were spend for medication and only less than 3% for monitoring systems.
Conclusion: The ROSSO is the first epidemiological cohort study, examining the longitudinal epidemiological data of the same patients over more than six years for type 2 diabetes mellitus in primary care practices. Stroke occurs more often than myocardial infarction, but even more patients suffer from congestive heart failure. Even in the first years after diabetes diagnosis an excess of costs for treating complications was observed. Costs for medication and monitoring systems represent only a small portion of the total costs.
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PS 17

Cytokines, chemokines and beta cell death

0414

The role of CBR1 in the pathogenesis of type 1 diabetes

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Background and Aims: Type 1 diabetes (T1D) is a genetically complex disease characterized by an immune-mediated destruction of pancreatic beta-cells. We have recently fine mapped and characterized a T1D linked region (containing 32 genes) on chromosome 21 (p=0.0002) in a Danish family material. Interesting candidate genes emerged within this region, and several have been described to have modulating effects during oxidative stress in cells. Of these, the carbonyl reductase 1 (*CBR1*) gene have additionally been demonstrated involved in regulation of apoptosis, with potential relevance for T1D pathogenesis. We have previously by two dimensional gel electrophoresis of IL-1 β exposed, diabetes-prone Bio-Breeding rat islets demonstrated that the CBR1 protein was down regulated fivefold by IL-1 β stimulation, and we have preliminary studies showing that the same is true on the mRNA level in human islets. The aim of the present study was to further characterize the *CBR1* gene and protein at functional/cellular levels.
Materials and Methods: Functional studies were performed in an insulinoma (INS-1) beta-cell line. Cells were stimulated with cytokines (IL-1β and/or IFN-γ) for different periods of time, as well as cultured under serum starving conditions. All conditions were analyzed in the absence or presence of a specific CBR1 inhibitor (Hydroxy-pp-met), previously reported to be able to inhibit serum starvation induced apoptosis in other cell types. Accumulated insulin content, nitric oxide (NO) generation and cellular viability (MTT) was measured for all conditions. Furthermore, apoptosis induction was estimated by a Caspase 3/7-assay and a cell death detection ELISA-assay. Cells treated with cytokines and serum starvation were evaluated using real time PCR and Western blot analyses for expression levels of mRNA and protein.
Results: Maximum down regulation of CBR1 on the mRNA level, was observed when INS-1 cells were stimulated with IL-1β (150 nM) for 12 hours. On the protein level we demonstrated a corresponding down regulation of the CBR1 protein level after 24 hours of IL-1β stimulation. We detected no differences in NO, insulin, MTT or Caspase 3/7 activity in cells stimulated with cytokines in absence or presence of the CBR1 inhibitor. However, we observed that serum starvation induced beta-cell apoptosis can be suppressed by addition of the CBR1 inhibitor.
Conclusion: In the current study we have demonstrated CBR1 expression to be regulated by IL-1β on mRNA as well as the protein level. This down regulation by IL-1β may be an important mechanism, potentially making beta-cells more vulnerable towards oxidative stress. Furthermore we demonstrated an apoptosis modulating effect of CBR1 in beta-cells subjected to serum starvation. However, more studies are in demand to clarify the exact function of CBR1

in beta-cells. Our findings support *CBR-1* as a potentially interesting candidate gene/protein in T1D.
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0415

Proteomic analysis of cytokine-induced dysfunction and death in insulin producing INS-1E cells

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Background and Aims: Type 1 diabetes is an autoimmune disease characterized by T-cell mediated destruction of the pancreatic beta-cells. Although the exact triggers initiating the disease need further unraveling, cell-destructive molecules, including cytokines (such as interleukin [IL]-1beta and interferon [IFN]-gamma) play a crucial role in beta-cell destruction. Previously performed time-course micro-array analysis of insulin producing INS-1E cells identified 698 genes as modified by cytokine exposure, many of which are related to apoptotic pathways. The aim of this study was to investigate the relevance of these findings at the protein level, as well as to detect translational and post-translational changes.
Materials and Methods: For this purpose quantitative changes in protein expression upon cytokine-treatment were investigated in INS-1E cells using 2-dimensional difference gel electrophoresis (2D-DIGE) and differentially expressed spots were identified by MALDI-TOF/TOF. INS-1E cells were incubated during 1, 4 and 24 hours with IFN-gamma (500U/ml) and IL-1beta (10U/ml). As expected, these cytokines induced nitric oxide (96 pmol/10^3 cells in cytokine-induced versus not detectable in control condition; n=4) and apoptosis (Annexin/PI staining; 35.2% increase compared to control condition; n=4 ($p<0.01$)). Differential protein expression was analyzed by 2D-DIGE, using 3 different fluorescent dyes (Cy2, Cy3 and Cy5). The samples were separated on 24 cm IPG strips in the first dimension (pH ranges 4–7 and 6–9) and run on a 12.5% polyacrylamide gel in the second dimension.
Results: Four independent experiments were performed, each detecting a total of approximately 2300 spots in the pH range 4–7 of which 107 spots were statistically significant regulated ($p<0.01$). For the pH range 6–9, approximately 1500 spots were detected, of which 36 altered expression ($p<0.01$). Nineteen differentially expressed spots have been identified up till now. The identified proteins can be classified in the following groups: 1) heat shock/chaperone proteins; 2) structural proteins; 3) proteins with various catalytic characteristics and 4) proteins with unknown or other characteristics. A comparison of this first set of differentially expressed proteins with the previously obtained micro-array data, revealed only 1 protein, ie. heat shock protein 70, as also being regulated at the mRNA level. Interestingly, another protein involved in ER stress, ie. BiP, was significantly altered in its level of phosphorylation (2.68-fold downregulation of the native protein, but a 3.63-and 2.72-fold upregulation of the one- and two-times phosphorylated forms, respectively ($p<0.01$)).
Conclusion: The present findings are complementary to the previously obtained micro-array data. A combination of the two techniques will provide a more detailed picture of the pathways involved in cytokine-mediated beta-cell destruction observed in type-1 diabetes.

0416

In Silico identification of NF-κB-regulated genes in pancreatic beta-cells

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Background and Aims: In type 1 diabetes mellitus (T1DM), pancreatic beta-cells are the target of an autoimmune-triggered destruction mediated in part by cytokines such as IL-1β and IFN-γ. These cytokines modify the expression of hundreds of genes, including genes related to stress responses and differentiated beta-cell functions. This leads to beta-cell dysfunction and death mainly by apoptosis. Several of these cytokine-induced genes are potentially regulated by the IL-1β-activated transcription factor NF-κB. Previous studies by our group have shown that cytokine-induced activation of NF-κB is mostly pro-apoptotic in beta-cells. We presently used a discriminant analysis-based approach to predict NF-κB responding genes on the basis of putative regulatory elements, to identify NF-κB-regulated gene networks in beta-cells.
Material and Methods: The top matching scores of locally over-represented transcription factor binding site (TFBS) matrices in proximal promoters of known NF-κB target genes were used to characterize upstream sequences (1000 bp) of NF-κB-regulated and non-regulated genes (120 genes each). These genes were used for training and evaluation of two supervised classification methods (Linear

and Quadratic Discriminant Analysis; LDA and QDA). The performance of the constructed discriminant classifiers was examined using a stratified cross-validation with an internal leave-one-out cross validation loop for automated feature selection and noise reduction. In order to predict potential NF-κB-regulated genes, the optimal discriminant classifier was used to analyse simultaneously the upstream sequences of rat and human beta-cell expressed genes and their homologous genes in other species. When possible, the reliability of the predictive results was assessed by comparison with available micro-array experimental data obtained by our group.

Results: As expected, 5 of the 6 TFBS matrices founded as enriched in the upstream sequences of known NF-κB-regulated genes correspond to transcription factors from the Rel/NF-κB family. Thus, each gene was described by a 30-element matrix matching scores vector. In the calibration phase of the analysis, we observed that LDA discriminated NF-κB-responding genes with 61% sensitivity, 91% specificity and 87% positive predictive value; LDA performed slightly better than QDA and was then used in the prediction phase. We identified 234, 253 and 583 NF-κB target genes in insulin producing INS-1 cells, primary rat beta-cells and human pancreatic islets, respectively. Predicted NF-κB targets show a significant enrichment in genes regulated by cytokines (IL-1β or IL-1β+IFN-γ), double stranded RNA and a viral infection, as compared to genes not regulated by these NF-κB-dependent stimuli (Fisher exact probability test ; $p<0.005$ in all comparisons). Since groups of genes subject to specific regulation are assumed to be evolutionary stable, we increased confidence in our predictions by selecting only genes with homologs predicted as NF-κB targets in other organisms, namely rat, mouse, human and chimpanzee.

Conclusion: This work allowed us to identify novel regulatory targets of NF-κB using a supervised classification method based on putative binding motifs. This provided new insights into the gene networks regulating cytokine-induced beta-cell dysfunction and death.

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0417

Cytokine-induced Fas and chemokine expression is inhibited by SOCS-3 in pancreatic β cells

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Background and Aims: Type 1 diabetes mellitus is a disease characterized by an autoimmune attack of the insulin producing β-cells in the pancreas. The pro-inflammatory cytokines IL-1β and IFN-γ are believed to be important effectors in the immune response leading to β-cell death. These cytokines can initiate the production of toxic free radicals and nitric oxide in the β-cell thereby directly causing its death. Moreover via activation of the NFkB and MAPK pathways they regulate the transcription of genes that may have deleterious effects in the β-cell. Among these is the gene encoding the pro-apoptotic receptor Fas as well as genes encoding various chemokines, many of these being NFkB dependent. Activation of the Fas receptor is initiated through binding to the Fas Ligand which is mainly expressed on the surface of activated T-cells and macrophages. The interaction results in apoptosis-mediated death of the Fas-expressing cell. Expression of chemokines enhances the inflammatory response as these proteins recruit activated immune cells. We have previously shown that the suppressor of cytokine signaling-3 (SOCS-3) can inhibit IL-1β and IFN-γ induced NO-production and apoptosis of the β-cell line INS-1. The **aim** of the present project is to investigate if SOCS-3 can influence cytokine induced Fas and chemokine expression in β-cells, as this could represent a new mechanism of SOCS-3 mediated protection of β-cells against inflammatory cytokines.

Materials and Methods: By means of a β-cell line with SOCS-3 inducible expression, it was investigated whether SOCS-3 influences IL-1β and IL-1β+IFN-γ induced Fas and chemokine mRNA production using real time RT-PCR analysis. Furthermore, the ability of SOCS-3 to influence the activity of a cytokine-responsive Fas promoter construct was measured by Luciferase analysis.

Results: IL-1β (150 pg/ml) induced a 7-fold increase in Fas mRNA expression after 6 hours of stimulation. This induction was inhibited by 40% in the presence of SOCS-3 ($p\leq0,05$). After 24 hours of stimulation the induction was reduced to 2-fold and not affected by SOCS-3. IL-1β+IFN-γ induced a 10-fold induction in the Fas mRNA expression after 6 hours and an 8-fold induction was seen after 24 hours. A reduction in the cytokine-mixture induced Fas mRNA expression by approximately 50% was seen both after 6 and 24 hours ($p\leq0,01$). The ability of SOCS-3 to influence a cytokine responsive Fas promoter was measured by Luciferase analysis. Transfected cells were exposed to IL-1β alone or to IL-1β+IFN-γ for 6 hours. SOCS-3 inhibited both IL-1β and IL-1β+INF-γ-induced activity of the Fas promoter by 30–40% ($p\leq0,05$). Moreover, preliminary data indicate that SOCS-3 is able to suppress cytokine induced chemokine mRNA expression in the β-cell.

Conclusions: The presented data indicate that SOCS-3 possibly via the blockade of the NFkB pathway can inhibit expression of the death receptor Fas, one of the major

apoptotic genes induced by cytokines in the β-cell. Furthermore preliminary data suggests that SOCS-3 can inhibit cytokine-mediated chemokine expression in a β-cell line. These data represent a new explanation of the protective effect seen by SOCS-3 in β-cells against cytotoxic cytokines.

0418

IL-1β+IFN-γ reduce anti-apoptotic phosphorylation of Bad, a proapoptotic Bcl-2 family member and induce cleavage of caspase-9 in the rat β cell line INS-1E

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Background and Aims: Interleukin (IL)-1β and Interferon (IFN)-γ are pro-inflammatory cytokines proposed to be effectors in β-cell destruction in Type 1 Diabetes. The Bcl-2 protein family encompasses important regulators of the mitochondrial (intrinsic) pathway that controls cleavage of caspase-9 leading to cleavage of caspase-3 and apoptosis. The family consists of both anti-apoptotic (e.g. Bcl-2 and Bcl-X_L) and pro-apoptotic members (e.g. Bid, Bax, Bak, and Bad). In β-cells, overexpression of Bcl-2 and Bcl-X_L inhibits the deleterious effects induced by cytokines and we have previously shown that IL-1β+IFN-γ increase the mRNA, but not protein expression of Bid, Bax, Bak, and Bcl-X_L. Neither Bcl-2 mRNA nor protein levels were modulated by cytokines. It remains to be clarified if cytokines change the activity of Bcl-2 proteins, e.g. by modulating the phosphorylation status. ***Aims:*** Since Bad in other cell types is a target of c-jun N-terminal kinase and Akt both of which are key signaling components in cytokine-induced β-cell death we investigated the effects of IL-1β+IFN-γ on Bad protein expression and phosphorylation. Further, the cleavage of caspase-9 and caspase-3 was investigated.
Material and Methods: Experiments were performed on the rat β-cell line INS-1E. Immunoblotting was performed to assess the protein expression and phosphorylation of Bad on Ser136 (anti-apoptotic phosphorylation) and cleavage of caspase-9 and caspase-3. The protein expression of Bad and cleavage of the caspases were investigated after exposure to IL-1β+IFN-γ for 4, 8, 12, and 24 hours and the phosphorylation of Bad on Ser136 was investigated after exposure to IL-1β+IFN-γ for 0.5, 1, 3, 6, 12, and 24 hours.
Results: Cytokines induced time-dependent de-phosphorylation of Bad on ser136, significant after 1 h. De-phosphorylation of Bad on Ser136 has been proposed to activate the pro-apoptotic activity of Bad by inhibiting 14-3-3 binding to Bad which under normal conditions retains Bad in the cytoplasm. By de-phosphorylating this residue, cytokines might induce a pro-apoptotic effect of Bad. To investigate this further we investigated if caspase-9 was cleaved as a measure of mitochondrial pro-apoptotic signaling and found that both caspase-9 and -3 were cleaved after 12 and 24 h of cytokine exposure. Furthermore, cytokines were found to decrease the protein expression of Bad by 20% after 12 hours of exposure. Since bad de-phosphorylation was observed prior to the reduction in protein expression we speculate that de-phosphorylation makes Bad more susceptible to degradation or negatively feeds-back on bad mRNA expression and/or protein synthesis.
Conclusion: Cytokines induced de-phosphorylation of Bad on ser136 followed by cleavage of caspase-9 and -3. Taken together, these data indicate that the mitochondrial pathway might be involved in cytokine-mediated β-cell death and that this process is mediated by activation of Bad.
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0419

Expression of a kinase deficient, dominant negative apoptosis signal-regulating kinase (ASK) 1 reduces cytokine- and endoplasmic reticulum stress-induced apoptosis in insulin-secreting INS-1 cells

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Background and Aims: Apoptosis of insulin-secreting pancreatic β-cells is the underlying cause of type 1 diabetes. Pro-inflammatory cytokines including interleukin (IL)-1β are thought to be primary immune mediators of β-cell destruction. In vitro, IL-1β signals β-cell apoptosis via the c-jun N-terminal kinase (JNK) stress signaling pathway. Further, recent evidence points toward endoplasmic reticulum (ER) stress being an essential pro-apoptotic signal in β-cells exposed to cytokines. However, the molecular mechanisms behind cytokine- and ER stress-induced β-cell apoptosis remain unclear. One potential mechanism is via apoptosis signal-regulating kinase (ASK) 1, known to mediate stress-induced JNK activation and cell death. Thus, the aim of this study was to examine if transfection with an expression vector encoding a kinase deficient (KD), dominant negative form of ASK 1 protects against cytokine- and ER stress-induced apoptosis in insulin-secreting cells.
Materials and Methods: Rat INS-1 cells were stably transfected with KD-ASK 1 or empty vector (mock). Apoptotic cell death was evaluated by detection of nucleosomes present in the cytoplasm by ELISA. JNK

activation was determined by immuno blotting using antibodies against the phosphorylated, activated forms of JNK1/2.
Results: Clones of INS-1 cells expressing KD-ASK 1 were less sensitive to IL-1β+interferon (IFN)-γ-induced apoptosis as compared to mock-transfected control cells. Further, apoptosis induced by thapsigargin (Tg), an inhibitor of sarco-endoplasmic reticulum calcium ATPase (SERCA) leading to ER stress, was reduced in cells expressing KD-ASK 1 compared to mock cells. Interestingly, neither cytokine-nor Tg-induced JNK phosphorylation was affected in cells expressing KD-ASK 1.
Conclusion: Our findings suggest that ASK 1 is involved in transducing cytokine-and ER stress-mediated apoptosis in insulin-secreting cells.

0420

The chemokine IP-10 as a mediator of decreased β cell mass in diabetes

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Background and Aims: Both type 1 and type 2 diabetes result from reduced β-cell mass and increased apoptosis, secretion of pro-inflammatory cytokines may contribute to the disease. The aim of this study was to investigate the role of the chemokine Interferon-gamma-inducible protein-10 (IP-10) on β-cell function and survival.
Materials and Methods: Isolated human islets were exposed to IP-10, β-cell apoptosis (by the TUNEL assay), replication (by Ki-67 staining) and glucose stimulated insulin secretion (GSIS) were measured. Additionally, cell viability was determined using the MTT colorimetric assay. IP-10 secretion and mRNA were analyzed in isolated islets from healthy organ donors and donors with type 2 diabetes.
Results: IP-10 secretion from diabetic islets was 825.55-fold and IP-10 mRNA was 33.33-fold increased, compared to control ($p<0.05$). Double staining for IP-10 and insulin in pancreatic sections from patients with type 2 diabetes revealed β-cells expressing IP-10, but no IP-10 staining was detected in control sections. 0.1 ng/ml IP-10 increased β-cell apoptosis 1.57±0.27-fold (n.s.) after 1 day and 2.78±0.55-fold ($p<0.01$) after 4 days of culture, compared to control.
Assessment of cell viability confirmed the proapoptotic effect of IP-10: Viabilities of cells treated with 0.1 ng/ml IP-10 for 1 day and 4 days were 91.52±1.98% and 85.33±2.34% of control value, respectively.
In parallel, GSIS was impaired in the IP-10 treated islets (1.48±0.37-fold stimulatory index vs. 3.54±0.35-fold in control, $p<0.01$) and insulin mRNA was 4-fold decreased. IP-10 also induced β-cell proliferation (1.5±0.47-fold at 0,1 ng/ml IP-10 and 2.71±0.61-fold at 50 ng/ml IP-10, compared to control). Western Blot analysis for p-Akt (Ser473) revealed Akt activation within 10 min. of addition of IP-10, together with cleavage of PAK-2, which would initiate apoptosis. Inhibition of the PI3K/Akt pathway by Wortmannin decreased IP-10 induced Akt activation as well as IP-10 induced apoptosis.
Conclusion: Our data suggest that IP-10 induced proliferation is mediated by Akt activation, but this finally leads to apoptosis. This is in line with previous data showing that proliferating β-cells are more susceptible to apoptosis. Thus, IP-10 possibly contributes to the impaired β-cell function and increased apoptosis in diabetes.

PS 18

Beta cell dysfunction and death in models of type 2 diabetes

0421

Altered autophagy in the pancreatic beta cells of human type 2 diabetes

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Background and Aims: Decreased beta-cell functional mass is crucial to the development and progression of type 2 diabetes (T2D). Although enhanced apoptosis is a well-known cause of increased beta-cell death, other mechanisms may play a role. Autophagy is the process which degrades long-lived proteins and cellular organelles, and may lead, if altered, to a form of programmed cell death morphologically distinct from apoptosis and necrosis.
Materials and Methods: We studied autophagic phenomena in the beta-cells from the pancreata of 18 multiorgan donors (8 non-diabetic, ND: age, 61±12 yrs; BMI, 25.2±2.5 Kg/m^2; and 10 with T2D: age, 67±8 yrs, BMI, 27.5±3.8 Kg/m^2).
Results: By electron microscopy 3708 cells were evaluated, and the analysis showed a slight, but significant decrease of beta-cells in T2D islets (62.0±2.2% vs 71.0±3.3%, $p<0.05$), with unchanged alpha- and delta-cell proportions. The percentage of dead cells was 14±2.4% in T2D and 5±1.4% in ND ($p<0.01$), with apoptotic cells representing 7±4% and 2±1.3%. respectively ($p<0.05$). The remaining proportions of

7±3% (in T2D) and 3±1% (in ND) ($p<0.05$) dead cells showed signs of autophagic cell death (membrane dysruption, extreme overload of vacuoles with/without electron-dense contents, no major chromatin condensation, no signs of inflammation). No cell with classical necrotic ultrastructure was found. In living beta-cells, the density volume (ml%) of autophagic vacuoles (containing still recognizable organelles, 0.6±0.1 vs 0.1±0.05) and of autophagosomes (containing lysosome degraded material, 7.7±1.1 vs 4.7±0.3) was significantly higher ($p<0.05$) in T2D than ND. Quantitative RT-PCR showed a significant ($p<0.01$) decrease of the expression (ng/gene over ng/beta-actin) of the lysosomal degrading enzyme cathepsin D (1.88±1.42 vs 4.45±2.29) in T2D islets versus ND, with unchanged levels of cathepsin F and H. These data were confirmed by microarray experiments performed on beta-cells captured by laser microdissection.

Conclusion: These results show that pancreatic beta-cells in human type 2 diabetes have signs of altered autophagy, possibly associated with reduced lysosomal activity, which may contribute to cause beta-cell functional and survival dysfunction.

0422

Oxidative stress in isolated human pancreatic islets: possible role of frataxin

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Background: Oxidative stress is a main mechanism leading to beta-cell damage in isolated islets. It has been suggested that the mitochondrial protein frataxin could be involved in the modulation of reactive oxygen and/or nitrogen species production. In fact, in animal models, disruption of the frataxin gene in pancreatic beta cells causes diabetes paralleled by an increase in reactive oxygen species. No information is currently available as for the role of frataxin in isolated human pancreatic islets.

Materials and Methods: We prepared human islets by enzymatic digestion and gradient separation from the pancreas of 18 non-diabetic (Ctrl, age 51.0±13.8 yrs: sex 12M/6F: BMI 24.2±3.7 kg/m2) and 10 type 2 diabetic (T2DM) multiorgan donors (age 64.3±7.6 yrs: sex 5F/5M: BMI 27.7±3.7 kg/m2), measured frataxin mRNA (by Real-time RT-PCR) and protein (by Western-blotting) expression, and assessed nitrotyrosine levels (by an ELISA technique) as marker of oxidative stress; finally, we silenced frataxin gene expression by siRNA transfection method.

Results: In T2DM, frataxin mRNA (−63.3±10.3%) and protein (−36.4±14.1%) expressions were significantly lower than in Ctrl. ($p<0.01$). Moreover, in T2DM there was an increase of nitrotyrosine levels (11.8±0.7 vs 7.5±0.4 nmol/l of Ctrl, $p<0.01$). When we silenced the frataxin gene in Ctrl (−36.3±10.1% of mRNA expression), we found a significant ($p<0.01$) increase of nitrotyrosine levels (+27.6±7.4%) and a reduction of insulin secretion (−25.4±3.3% of S.I. ctrl), respect to non-transfected islets ($p<0.01$).

Conclusion: These results show a reduced expression of frataxin gene in T2DM islets, and indicate that a reduction of frataxin gene expression is associated with an increase of oxidative stress and reduced insulin secretory function. These data suggest a physiological role of frataxin in the modulation of human beta-cell function, and may be of help in developing strategies to improve survival of isolated islets.

0423

The role of hyperglycaemia in dexamethasone-induced apoptosis in insulin secreting INS-1 cells

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Background and Aims: Glucocorticoids are known to induce apoptotic cell death in a variety of cells. In lymphocytes, glucocorticoids increase cytosolic Ca^{2+} by activation of Ca^{2+} release from IP3-sensitive intracellular stores. Ca^{2+} together with heat shock protein 90 (HSP90), released from the stimulated glucocorticoid receptor (GR), activates calcineurin (PP2B). Calcineurin activation contributes to mitochondrial mediated apoptosis by dephosphorylation of BAD. The aim of the present study was to examine whether activation of PP2B and dephosphorylation of BAD are involved in dexamethasone (dex)-induced apoptosis, and whether hyperglycaemia potentiates the cytotoxic effect of dex in insulin secreting cells.

Materials and Methods: INS-1 cells were cultured under standard conditions in the presence of different glucose concentrations (2, 5, 11 and 20 mM). Apoptosis was quantified by DAPI-stained condensed nuclei and by TUNEL assay. Calcineurin activity was measured using pNPP (p-nitrophenylphosphate) or ^{32}P-RII peptide as substrate. INS-1 cytosolic Ca^{2+} activity was measured using the fluorescent Ca^{2+}-indicator Fura-2AM. BAD phosphorylation was quantified by Western blotting.

Results: The synthetic glucocorticoid dex (100 nM) induced apoptosis in insulin secreting cells. RU486 (1 μM), the glucocorticoid receptor (GR) antagonist, inhibited the dex effect. Two PP2B inhibitors, FK506 (0.1 μM) and deltamethrin (1 μM) reduced dex-mediated apoptosis by 34% and 72%, respectively. The rate of dex-induced apoptosis was significantly higher at 20 mM (10.6%) than at 2 mM glucose (4.2%). Dex did not increase cytosolic Ca^{2+} activity while carbachol and glucose exerted their known effects. PP2B activity was significantly increased by dex in cells cultured at 11 and 20 mM glucose, whereas dex did not enhance the enzyme activity at 2 and 5 mM glucose. Thus, stimulation of PP2B by dex was glucose-dependent. Geldanamycin, an antagonist of HSP90, inhibited stimulation of PP2B by 80% in INS1 cells cultured at 11 mM glucose. Dex decreased phosphorylation of BAD.
Conclusion: These data suggest that dex-induced apoptosis in INS-1 cells involves PP2B stimulation by HSP90 released from the stimulated GR complex and is enhanced by hyperglycaemia, probably through glucose-stimulated Ca^{2+} entry.

0424

The role of renin-angiotensin system blockers in the protection of pancreatic beta cell function in diabetic rats

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Background and Aims: Many large-scale clinical studies have shown that angiotensin converting enzyme inhibitor (ACEI) and AT_1 receptor blocker (ARB) is associated with a low incidence of new-onset diabetes among high risk patients. This study aimed to determine if the block of local renin–angiotensin system by ACEI and ARB play a role in the protection of pancreatic β-cell function in type 2 diabetes rats.
Materials and Methods: Type 2 diabetic rat models were induced by intraperitoneal injection of a small dose of streptozotocin plus high fat high caloric laboratory chow. The diabetic rats were randomly divided into diabetic group, ACEI group and ARB group. 10 weeks treatment of perindopril or valsartan was given to ACEI group or ARB group. After an overnight fasting, intravenous glucose tolerance testing and insulin release testing were performed to assess the first-phase insulin response in diabetic rats. Serum glucose was analyzed by the glucose oxidase method. Plasma insulin was measured using radioimmunoassay. Gene expression of AGT, insulin, TGF-β_1 in pancreas extracts was determined using semiquantitative RT-PCR. We performed dual staining for proliferating cell nuclear antigen (PCNA) and insulin to assess intraislet proliferation, transferase-mediated dUTP nick-end labeling staining (TUNEL) to assess islet cell apoptosis, and staining for transforming growth factor-β1 (TGF-β1) as well as type I and type IV collagen to assess islet fibrogenesis.
Results: Both perindopril and valsartan treatment could significantly improve first-phase insulin secretion in diabetic rats ($P<0.05$). Compare with diabetic group, staining density for insulin in ACEI group and ARB group was increased by 45%, 37% respectively. Intraislet cell death, measured by TUNEL staining, was significantly decreased by 64% in islet of ACEI group, and by 52% in ARB group compared to diabetic group. The number of cells staining positively for PCNA was increased by 51% and 47% respectively in ACEI and ARB group. And the incremental area under the insulin curve between 0 and 10 min was negatively correlated with the islet cell apoptosis, and positively correlated with islet cell proliferation. TGF-β1 mRNA and protein expression as well as type I and type IV collagen staining were decreased by 39% and 26% respectively in two groups with treatment. Gene expression of AGT was much greater in diabetic islet compared with control group, and both treatments had no significant effect on its expression.
Conclusion: In type 2 diabetic rats, local RAS block by ACEI and ARB could decrease islet β-cell apoptosis, increase hyperplasia, and decrease the islet fibrogenesis. Long-term treatment of ACEI and ARB could improve pancreatic β-cell architecture and increase the first-phase insulin secretion in type 2 diabetes. These findings support that local RAS block plays an important role in the protection the pancreatic β-cell architecture and function in type 2 diabetes.
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0425

Hepatocyte growth factor (HGF) protects pancreatic beta cells from fatty acid-induced apoptosis

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Background and Aims: Type 2 diabetes is characterized by peripheral insulin resistance, pancreatic β-cell dysfunction and decreased β-cell mass with increased rates of beta cell apoptosis. Prolonged exposure to high level of free fatty acids (FFAs) has detrimental effects on β-cell function and survival. Hepatocyte growth factor (HGF) is a mesenchyme-derived multifunctional protein that plays a critical role in cell expansion and cell survival in various eukaryote cell types. While the action of HGF on the proliferation and differentiation of pancreatic beta cells is well documented, its role in antagonizing beta cell death in response to pro-apoptotic agents is not fully characterized.
Materials and Methods: Rat insulin-producing cell RINm5F were cultured and exposed to FFA (long-chain fatty acid oleate:palmitate 2:1) for 72 h in order to induce apoptosis.
Results: In the present study we demonstrated that HGF inhibits FFA-induced apoptosis of cultured beta cells, reducing DNA fragmentation and improving cell survival. This anti-apoptotic action of HGF was mediated by the inhibition of oxidative stress that occurs in response to the prolonged exposure to high FFAs. HGF inhibited the formation of Reactive Oxygen Species and their negative effect on cell survival. The beneficial effect of HGF was mediated by an increase in bcl-2 level (mRNA and counterpart protein) and activity. Furthermore, investigation of the signaling pathway known to be activated HGF and to be associated with the regulation of bcl-2 showed that the protective effect of HGF required the phosphorylation of PI3K.
Conclusion: The present study demonstrates that HGF protects beta cells from the pro-apoptotic action of FFAs, inhibiting mitochondrial damage and ROS production. The cell survival action of HGF occurred via a PI3K-dependent signaling pathway.
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0426

PPARalpha plays a role in glucose stimulated insulin secretion and protects against lipotoxicity in INS-1E beta cells

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Background and Aims: Among the molecular targets for the treatment of obesity and diabetes, great attention has been given to the three peroxisome-proliferator-activated receptor (PPAR) subtypes, nuclear lipid sensors, able to modulate metabolic events by regulating gene expression. In particular, PPARalpha plays a crucial role in metabolic adaptation by inducing key genes for fatty acid oxidation and ketogenesis in mitochondria, with a protective role under conditions of fatty acids challenge. Regarding beta-cells, PPARalpha function remains poorly investigated and controversial. In insulinoma cells overexpressing PPARalpha, glucose-stimulated insulin secretion (GSIS) was reported to be either reduced (J. Lipid Res. 2002, 43:936–43.) or potentiated (Endocrinology 2005, 146: 3266–3276). The aim of our project was to investigate the molecular basis of apparent opposite effects of PPARalpha in insulin secreting cells. Specifically, we assessed the relative contributions of PPARalpha over-expression and fatty acid exposure to metabolism secretion coupling,
Materials and Methods: We transduced INS-1E beta-cells with adenoviruses expressing respectively PPARalpha and RXR, and then performed experiments 1 day and 3 days after transduction. Cells were cultured either in normal complete RPMI-1640 media or supplemented with 0.4 mM oleate. At the end of the culture periods, we measured mitochondrial membrane potential (rhodamine-123 fluorescence), cellular calcium levels (Fura-2 fluorescence), and insulin secretory responses (analysed by radioimmunoassay) to stimulatory glucose concentrations.
Results: PPARalpha over-expression increased GSIS 1 day after transduction in normal conditions (+26%) and did not affect the glucose response in oleate-treated cells. Interestingly, 3 days after transduction, PPARalpha increased GSIS in the presence of oleate (+153%), showing strong protective effects against fatty acid induced toxicity. In order to understand the mechanisms implicated in PPARalpha mediated increase in GSIS, we quantified the mitochondrial membrane potential under the same conditions. After 1 day, mitochondrial hyperpolarization upon 15 mM glucose stimulation was blunted in oleate-cultured cells (−21%), an effect reinforced by PPARalpha over-expression (−46%). No effects of PPARalpha were noticed in the absence of oleate. After 3 days, mitochondrial membrane potential was not affected by PPARalpha in normal conditions, but dramatically reduced in the presence of oleate, with or without PPARalpha over-expression (−42% and −80% respectively). Potassium-induced calcium rises were blunted in the presence of oleate and neither protected nor aggravated by PPARalpha over-expression.
Conclusion: These data show complex relationship between the transcription factor PPARalpha and the nutrient state of cultured cells. Specifically, PPARalpha over-

expression protected INS-1E beta-cells against oleate induced lipotoxicity. Current experiments measuring beta-oxidation versus glucose oxidation, together with PPAR-alpha target gene expression, should help understanding how lipid and glucose metabolisms interact with the control of insulin secretion.
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0427

Protect effects of fenofibrate and pioglitazone on islet β cells of rats by corrected inflammatory and apoptotic regulation

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Background and Aims: To explore the expressions of PPAR-α, PPAR-γ, inflammatory mediators, apoptotic regulator caspase-3, and apoptotic level in pancreatic islets by establish obese rat models induced by high-fat or high-sucrose diets. To investigate the effects of PPAR-α agonist fenofibrate and PPAR-γ agonist pioglitazone on function and apoptosis of islets.
Materials and Methods: S-D obese rat models were established with high- carbohydrate diet (high-sucrose diet (CO group), high carbohydrate diet with fenofibrate (CF group) or pioglitazone (CF group) and high-fat diet high-fat diet (FO group), high-fat diet with fenofibrate (FF group) or pioglitazone (FP group), while the control rats were with normal diet (control group). Duration of intervention were 8–10 weeks,. Immunohistochemistry and western blot were performed to evaluate the expressions of insulin, glucagons, Somatostatin, PPAR-α, PPAR-γ, nuclear factor kappa-B (NF-Éb), inhibitory protein κB (I-κB), P38, extra cellular signal-regulated kinases1 (ERK1) and caspase-3 proteins in islets, in situ hybridization and RT-PCR were underwent to access the mRNA levels of PPAR-α, PPAR-γ, NF-κB and caspase-3. At the same time, apoptotic level were evaluated by TUNEL, and islets mass were scored in tissue slides.
Results: 1) Islets mass enlarged in CO and FO groups. The compositions of islet cells were as same as the control. The expression of insulin was lower in CO and FO groups than the control, but after using pioglitazone, less islets mass and more insulin expression were found in CP and FP groups. 2) Comparing with the control group, expressions levels of PPAR-α, PPAR-γ protein and mRNA were reduced in CO and FO group, and the expression of PPAR-α protein and mRNA increased in CF and FF groups, the expression of PPAR-γ protein and mRNA elevated in CP and FP groups. 3) The levels of NF-κB, P38, ERK1 proteins and mRNA increased significantly in FO group, the expressions of NF-κB, P38 decreased in FF and FP groups, the level of ERK1 only decreased in FP group. the protein level of I-κB was no difference among control, CO and FO group, and I-κB increased in CF and FF groups. 4) In control group, the expression of caspase-3 was lowest, and elevated gradually in CO and FO groups, the level of caspase-3 decreased in CF, CP, FF and FP groups comparing with CO and FO groups. Few TUNEL-position cells were found in all the groups.
Conclusion: In the obese rat models induced by high-carbohydrate and high-fat diets, islets mass enlarged, but insulin level decreased; The expressions of PPAR-α and PPAR-γ in islets decreased significantly, and the level of several inflammatory mediators and caspase-3 increased in islets of obese rat model. Though no evidence of inflammatory response and apoptosis were detected, the dysfunction of inflammatory and apoptotic redulators already existed in islets of obese rats. Fenofibrate and pioglitazone could protect βcells function and survival by corrected inflammatory and apoptotic regulation.

0428

Expression of islet inducible nitric oxide synthase and inhibition of glucose stimulated insulin release after long-term lipid infusion in the rat is counteracted by PACAP27

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Background and Aims:Chronic exposure of pancreatic islets to elevated plasma lipids (lipotoxicity) can lead to β-cell dysfunction. We examined, whether the expression of islet inducible nitric oxide synthase (iNOS) and the concomitant inhibition of glucose-stimulated insulin release seen after lipid infusion in rats was modulated by the islet neuropeptide PACAP27.
Materials and Methods:Confocal microscopy, HPLC and RIA were used.
Results: Lipid infusion for 8 days induced a strong expression of islet iNOS, which was mainly confined to β-cells, and was still evident after incubating islets at 8.3 mmol/l glucose. This was accompanied by a high iNOS-derived NO generation, a decreased insulin release and increased cyclic GMP accumulation. No iNOS expression was found in control islets. Addition of PACAP27 to incubated islets from lipid-infused rats resulted in loss of iNOS protein expression, increased cAMP, reduced cGMP and suppression of the activities of neuronal constitutive NOS (ncNOS) and iNOS and and enhanced glucose-stimulated insulin response. These effects

were reversed by the PKA inhibitor H-89. The suppression of islet iNOS expression induced by PACAP27 was not affected by the proteasome inhibitor MG 132, which by itself induced the loss of iNOS protein making a direct proteasomic involvement unlikely.
Conclusion:Our results suggest that PACAP27 through its cAMP and PKA-stimulating capacity strongly suppresses not only ncNOS but importantly also the lipid-induced stimulation of iNOS expression by a non-proteasomic mechanism. Thus PACAP27 restores the impairment of glucose-stimulated insulin release and additionally might induce cytoprotection against deleterious actions of iNOS-derived NO in β-cells.

0429

Hyper-activation of liver X receptor suppresses cell proliferation and induces apoptosis in pancreatic beta cells
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Background and Aims: Liver X receptors (LXRs, alpha and beta isoforms) regulate cholesterol, lipid and glucose metabolism. Pancreatic beta-cells express only the LXRbeta. LXRs form functional heterodimers with the retinoid X receptors (RXR). We showed before that activation of LXR or LXR/RXR heterodimer modulates insulin secretion and biosynthesis via regulation of glucose and lipid metabolism in MIN6 cells and isolated pancreatic islets. In this study we investigated the effects of the LXR agonist TO901317 and the RXR agonist 9-cis-retinoic acid (9cRA) on cell proliferation and apoptosis in mouse insulinoma MIN6 cells.
Materials and Methods: Effects of TO901317 and 9cRA on cell proliferation in MIN6 cells were measured with a BrdU incorporation assay and flow cytometry. Apoptosis in MIN6 cell and isolated pancreatic islets was assessed with caspase assay, DNA fragmentation ELISA and annexin V assay.
Results: While the LXR agonist TO901317 shows no effect on proliferation of MIN6 cells, combination of T0901317 with 9cRA strongly inhibits cell proliferation. Flow cytometry analysis of cell cycle in cells treated with the compounds demonstrated that activation of LXR/RXR prevented cells from G0/G1 to G2 phase progression. Furthermore, combination of T0901317 and 9cRA produced a higher apoptosis rate and increased caspase 3/7 activity in MIN6 cells. Activation of neither RXR nor LXR alone was sufficient to bring the cells into an apoptotic state. However, LXR or LXR/RXR activation significantly increased the susceptibility of MIN6 cells to free fatty acid- and cytokine- induced apoptosis. In isolated rat islets the apoptotic effect of palmitic acid was dramatically increased with TO901317 alone and even more with the combination of TO901317 and 9cRA.
Conclusion: Activation of LXR/RXR heterodimers inhibits cell proliferation and induces apoptosis in pancreatic beta-cells. Activation of LXR with T0901317 makes the beta-cell more sensitive to free fatty acid-induced apoptosis.

PS 19

Beta cell damage and apoptosis

0430

Effects of IP_3R and RyR Ca^{2+}-release channels on beta cell ER-stress
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Background and Aims: The endoplasmic reticulum (ER)-stress response has been implicated in multiple animal models of diabetes and several forms of the human disease. This cellular response can be caused by blocking ER Ca^{2+} uptake at the SERCA pump. However, the effects of modulating ER Ca^{2+} release on this cell death pathway remain unclear. In the present study, we examined the effects of stimulating or inhibiting two classes of ER-resident Ca^{2+} channels, namely the inositol 1,4,5 trisphosphate receptor (IP_3R) and the ryanodine receptor (RyR), on lumenal ER-Ca^{2+} dynamics, the induction of ER-stress and β-cell apoptosis.
Materials and Methods: The glucose-responsive MIN6 β-cell line has previously been used to model ER-stress and was used in this study. ER-stress and apoptosis was initiated with the commonly used SERCA inhibitor, thapsigargin and detected by western blot using antibodies to CHOP/GADD153 and cleaved caspase-3. IP_3R were inhibited using xestospongin C and activated following stimulation with carbachol. RyR were inhibited using high micromolar concentrations of ryanodine and activated with low-dose ryanodine or caffeine. Luminal Ca^{2+} levels in the ER were monitored in real-time using fluorescence resonance energy transmission (FRET) microscopy and the recently developed D1ER cameleon. Cell death was also quantified in real-time over a period of up to 48 hours by monitoring cellular propidium iodide incorporation using an automated Cellomics imaging system.
Results: First, we examined whether disrupting Ca^{2+} homeostasis by blocking ER Ca^{2+}-release channels in the

absence of SERCA inhibition would be sufficient to induce ER-stress. Although ryanodine is known to induce an alternate mode of programmed cell death in β-cells, neither 10–100 μM ryanodine nor 10 μM xestospongin C, alone or in combination, led to a robust increase in CHOP expression. As a positive control, 1 μM thapsigargin decreased luminal ER Ca^{2+} and induced marked ER-stress, caspase-3 activation and cell death (propidium iodide-positive β-cells). Next, we sought to determine whether inhibiting Ca^{2+} release via IP_3R and RyR could blunt the ability of thapsigargin to deplete the ER Ca^{2+} and induce ER-stress. In these experiments, inhibiting Ca^{2+} release through either channel led to a delay in thapsigargin-induced ER-stress and cell death. Finally, we questioned whether stimulating Ca^{2+} release through IP_3R or RyR would further deplete ER Ca^{2+} and accelerate ER-stress in the presence of thapsigargin. 100 μM carbachol evoked a robust decrease in ER Ca^{2+} and potentiated the effects of thapsigargin on ER-stress and cell death.

Conclusions: The present study illustrates the interactions between SERCA, IP_3R and RyR in the control of ER Ca^{2+} levels. All three components of the ER Ca^{2+} handling machinery can regulate distinct cell death pathways, although among these only SERCA-dependent depletion of the ER is sufficient to induce robust β-cell ER stress. Together, these studies indicate that β-cell ER-stress can be modulated at the level of the Ca^{2+} release channels, particularly the IP_3R.

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0431

FGF-21 is expressed in pancreatic islets and stimulates beta cell function and survival

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Background and Aims: We have recently demonstrated that fibroblast growth factor 21 (FGF-21) stimulates glucose uptake in adipocytes and normalizes plasma glucose levels in diabetic animal models. In this study we examined expression and effects of FGF-21 in pancreatic beta-cells.

Materials and Methods: RT-PCR was used to examine expression of FGF-21 in islets and cell lines. BrdU proliferation ELISA, caspase assay and DNA fragmentation assay in INS-1E cells and isolated islets were used to assess effect of FGF-21 on beta-cell growth and survival. Insulin secretion and insulin content was measured in INS-1E cells and islets from healthy rodents or diabetic GK rats and *db/db* mice.

Results: FGF-21 expression could be detected in rodent islets, purified beta-cells as well as insulinoma cell lines. In rat islets and INS-1E cells FGF-21 induced phosphorylation of the adapter protein FRS2alpha as well as activated Erk1/2 and Akt signaling pathways. While FGF-21 had no effect on proliferation of INS-1E cells, it could rescue cytokine treated cells from apoptosis. Treatment of isolated islets from healthy animals for 4–72 h with FGF-21 produced an increase in islet insulin content and islet insulin mRNA levels, while no significant effect of FGF-21 treatment on insulin secretion was observed. The FGF-21 induced increase in insulin production was blocked with a selective Erk1/2 inhibitor. Treatment of isolated islets from diabetic animals with FGF-21 produced increases in both insulin secretion and islet insulin content. Moreover, infusion of FGF-21 to *db/db* mice normalized plasma glucose levels. Insulin secretion and insulin content measured in islets isolated from FGF-21 treated mice were significantly elevated.

Conclusion: FGF-21 activates Erk1/2 and Akt signaling cascades in pancreatic beta-cells. FGF-21 increases insulin biosynthesis and protects the beta-cell from cytokine induced apoptosis. FGF-21 can lower plasma glucose in diabetic animals at least partially via its effects on pancreatic beta-cell function.

0432

Overexpression of IA-2 induces G2/M arrest and apoptosis in beta cells

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Background and Aims: IA-2 is a major autoantigen in type 1 diabetes. Up to 70% of newly diagnosed patients have autoantibodies to IA-2. These autoantibodies appear years before the development of clinical disease and in individuals with autoantibodies to both IA-2 and glutamic acid decarboxylase (GAD65), the likelihood of developing type 1 diabetes within 5 years is 50% or greater. In spite of the existence of autoantibodies, it is still unclear whether IA-2 itself is involved in beta cell destruction. To address this question, we overexpressed or knocked down IA-2 expression in MIN-6, mouse beta cell line, and we investigated the role of IA-2 in beta cell destruction.

Materials and Methods: We established permanent IA-2 transfected MIN-6 cell lines with G418 resistant gene. IA-2 expression also was knocked down with IA-2 siRNA. Apoptosis was detected by flowcytometry, Western blot and DNA fragmentation analysis, and cell cycle also was determined by flowcytometry analysis. Phosphorylation of

PDK-1 and the proteins of Akt signaling was analyzed by Western blot analysis.
Results: Overexpression of IA-2 puts MIN-6 beta cells into a pre-apoptotic state and exposure to a high concentration of glucose results in G2/M arrest and apoptosis. Treatment of the cells with IA-2 siRNA prevents both G2/M arrest and apoptosis. Analysis of the molecular events involved in pre-apoptotic cells revealed a decrease in phosphorylation of PDK-1 and the proteins of the Akt signaling pathway. Treatment of the cells with IA-2 siRNA increased Akt phosphorylation in both the IA-2 transfected pre-apoptotic cells and the non-transfected cells. In addition to being more susceptible to glucose-induced apoptosis, IA-2 pre-apoptotic cells are more susceptible to cytokine (TNF-alpha and INF-gamma) induced apoptosis.
Conclusion: Viewing the IA-2 system as a model, our studies suggest that overexpression of other still unidentified host proteins may put cells into a pre-apoptotic state which upon exposure to environment signals could trigger apoptosis and beta cell destruction.

0433

SHIP2 inhibition improves insulin signal transduction in insulin producing INS1E cells: Potential anti-apoptotic effects?

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Background and Aims: The lipid phosphatase SH2-containing inositol phosphatase 2 (SHIP2) is expressed in insulin target tissues, and functions to dephosphorylate the second messenger phosphatidylinositol-3,4,5-trisphosphate at the 5′-position. Polymorphisms of the SHIP2 gene promoter which lead to increased SHIP2 expression are associated with Type 2 diabetes. Adenoviral overexpression of a dominant negative SHIP2 mutant (dnSHIP2) improves insulin signaling and glucose uptake in differentiated L6 myocytes and 3T3-L1 adipocytes. Furthermore, liver specific expression of dnSHIP2 in db/db mice decreases fasting glucose levels. Here we describe for the first time the effects of SHIP2 inhibition in INS1E insulinoma cells.
Materials and Methods: C3A hepatoma cells or INS1E insulinoma cells were infected with recombinant adenoviruses expressing either β-galactosidase (β-Gal) or dnSHIP2 at a multiplicity of infection (MOI) of 25 and incubated for 40 h (C3A) or 24 h (INS1E). Following serum free preincubation for 2 h, cells were incubated for 15 min with insulin (0.1 nM to 1 μM). Phosphorylation of Akt was detected by phospho-Akt-ELISA or by Western blot. Furthermore GSK3α/β phosphorylation and Bad phosphorylation was measured by Western blot.
Results: To validate the functionality of the adenoviruses expressing β-galactosidase (β-gal) or dnSHIP2 we infected C3A hepatoma cells with both viruses and analysed the phosphorylation of Akt in response to insulin. By using a phospho-Akt ELISA we showed that overexpression of dnSHIP2 shifted the insulin dose response curve to the left and significantly increased the maximum insulin effect. Consistent with the literature that human islets express SHIP2, we detected significant SHIP2 expression in the INS1E insulinoma cell line. Adenoviral infection resulted in>90% infection of INS1E cells and in a 12fold over-expression of dnSHIP2 over the endogenous protein. Overexpression of dnSHIP2 was accompanied by a significant increase in Akt phosphorylation under basal and insulin-stimulated conditions, as detected by phospho-Akt-ELISA. Similarly, we showed an increased basal and insulin stimulated phosphorylation of GSK3α/β in INS1E cells following SHIP2 inhibition. Because increased Akt activity is directly linked to increased Bad phosphorylation and therefore to inhibition of apoptosis we analysed Bad phosphorylation after dnSHIP2 expression. As expected, the phosphorylation of Bad is significantly increased in comparison to the β-Gal expressing control.
Conclusion: These data show for the first time that dnSHIP2 acts as an insulin sensitizer/mimetic in in INS1E insulinoma cells with respect to early and also downstream insulin targets, namely Akt, Bad and GSK3 phosphorylation. Our data raise the interesting possibility that SHIP2 inhibition not only has an effect on glucose uptake in liver and muscle, but may also potentially exert important anti-apoptotic effects in beta-cells.

0434

Inducible nitric oxide synthase-nitric oxide plays an important role in hypoxic injury to pancreatic β cells

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Background and Aims: Islet transplantation is a potential strategy to cure type 1 diabetes mellitus. However, two or more donors are usually needed for one recipient because a substantial part of the graft becomes nonfunctional due to several factors including hypoxia. Though hypoxic exposure to pancreatic β cells has been reported to induce apoptotic cell death, the molecular processes involved in hypoxia-related cell death are poorly understood. Hypoxia is known to induce inducible nitric oxide synthase (iNOS)-derived

nitric oxide (NO) production in many types of cells. The aim of the study was to investigate iNOS-NO signaling and its role in apoptosis in pancreatic β cells under hypoxic conditions.

Materials and Methods: Mouse insulinoma cells (MIN6) and primary isolated islets from Sprague-Dawley rats were incubated in an anaerobic chamber for up to 12 hours. Cell viability was measured by acridine orange/propidium iodide staining. Caspase-3 activation was also determined. NO release into culture medium was measured using a Griess reagent. The expression of iNOS mRNA and protein was examined using real time RT-PCR and Western blot analysis.

Results: Marked cell death (~50%) occured within 6 hour after hypoxic exposure to MIN6 and rat islets. Immunoreactivity to activated caspase-3 was observed. NO production was increased in a time dependent manner. Expression of iNOS mRNA and protein was significantly increased at 4 and 6 hour after hypoxia. iNOS expression was confirmed by immunostaining. Of note, Pdx-1 mRNA expression was markedly attenuated by hypoxia. The Pdx-1 gene promoter activity in MIN6 was negatively regulated by hypoxia or a NO donor, DETA-NO. Pretreatment with a selective iNOS inhibitor, 1400W, significantly prevented apoptotic cell death by hypoxia.

Conclusion: Our data suggest that iNOS-NO plays an important role in hypoxic injury to pancreatic β cells. Therefore, iNOS-NO might be a potential therapeutic target for improving engraftment of transplanted islets.

0435

Controlled over expression of uncoupling protein 2 in INS-1 cells exerts minor effects on beta cell function and does not protect against cytokine-induced toxicity

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Background and Aims: Uncoupling protein 2 (UCP-2) is proposed to have diabetogenic influence by decreasing efficiency of glucose metabolism to generate ATP and/or other signaling molecules for insulin secretion. However, UCP-2 may also exert a beneficial role, coupled to protection against radical oxygen formation (ROS). Studies in knockout or un-restricted overexpression models have given evidence for both the afore-mentioned negative and positive effect; however these extremes of regulation make interpretations difficult in physiological terms.

Material and Methods: We transfected INS-1 cells to obtain a tet-on, UCP-2 inducible cell line. Induction by 0.1 or 1.0 μg/ml doxycyclin for 48 h increased UCP-2 protein by respectively 178±29 and 424±113%.

Results: Mitochondrial membrane potential, ATP and insulin contents were not significantly changed. Induction by 0.1 μg/ml of doxycyclin failed to affect oxidation of C14 glucose and C14-oleate. Glucose (27 mM)-induced insulin secretion tended to be reduced in induced cells, $p<0.08$. Secretion at 3.3 mM glucose+/− 20 mM KCl was not affected. A protective effect against ROS was tested for H2O2 and interleukin -1 beta (IL-1) by MTT assay. H202 exerted protection after induction with 1 μg/ml of doxycycline ($p=0,01$) but not after 0.1 μg/ml. No degree of induction prevented a decrease of MTT after 24 h exposure to IL-1. Instead, combined exposure to 0.2 mM oleate and 500 ng/ml IL-1b aggravated a reduction of MTT ($p=0,02$).

Conclusion: Controlled, moderate over expression of UCP-2 neither exerts major negative effects on beta cell function nor generalized protective effects against ROS. Thus, the functional role of UCP-2 may be mostly a permissive one.

0436

Search for molecules involved in the increased vulnerability of adult β cells after a maternal low protein diet. Prevention by taurine

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Background and Aims: We have shown that low protein diet induces long-term consequences on the endocrine pancreas of the progeny. Islets of adult rats that received a low protein diet during gestation and lactation but a normal diet after weaning, maintained a high vulnerability toward cytokines already observed at fetal age. Taurine is an amino acid of which concentration was reduced in the plasma of both mothers and fetuses fed a low protein diet. When malnourished dams are supplemented with taurine, a normal sensitivity toward cytokines was restored in the islets of the adult progeny.

Cytokines like IL-1β, TNF-α or INF-γ enhance the expression of genes like iNOS (inducible nitric oxide synthase), or chemokines such as MCP-1 (monocyte chemoattractant protein-1) and IP-10 (interferon inducible protein-10) in β cells. Their products are known to contribute to β cell death in type-1 diabetes.

	C		R		CT		RT	
	No Cytokines	Cytokines	No Cytokines	Cytokines	No Cytokines	Cytokines	No Cytokines	Cytokines
Apoptosis (%)	0.5±0.1	2.0±0.3	1.6±0.4*	3.3±0.3*	1.1±0.2	3.5±0.6°	0.7±0.1#	2.9±0.2
NO (pmol/islet/ 24 h)	9.6±3.4	57±5.8	50±11***	92±10.4*	21±5.0	85±8.3°	16.4±6.4##	80±7.4

Effect of maternal diet and early taurine supplementation on adult islet apoptosis and NO secretion.
* p<0.05 R vs. C, *** p<0.001 R vs. C, o p<0,05 CT vs. C, # p<0.05 RT vs. R and ## p<0.01 RT vs. R.

Our aim is to determine if the expression of these molecules is modulated by early low protein diet and taurine supplementation.

Materials and Methods: Wistar rats were fed with a 20% (Control [C group]) or an isocaloric 8% protein diet (Recovery [R group]) supplemented or not with taurine (CT and RT group) during gestation and lactation. After weaning, offspring received a 20% protein diet. Islets from 3 month-old rats were isolated and cultured. On day 3, they were incubated or not with cytokines (IL-1β 50U/ml, TNF-α 100 U/ml and INF-γ 100U/ml) for a 24 h period. Apoptotic rate was analysed with TUNEL method. NO, MCP-1 and IP-10 secreted by islets were quantified.

Results: Compared to controls, islets from R group featured an enhanced apoptotic rate and secreted more NO. Both parameters increased with cytokines and remained higher in the R group. Maternal taurine supplementation to the LP diet normalized both apoptotic rate and NO secretion in the RT islets cultured in normal medium. In the presence of cytokines, the rate of apoptosis and NO production were lower in the RT group compared to the R group so that the difference between C islets and RT islets was no more significant. However, taurine added to the dam's control diet caused an increase in both apoptosis and NO secretion when the islets were cultured with cytokines. Neither the LP diet nor taurine affected the MCP-1 and IP-10 production, which were only released in the presence of cytokines.

Conclusion: The increased apoptotic rate in the adult islets of early malnourished rats may be partially due to an increase in NO production. There was no evidence that maternal malnutrition interfered with mechanisms leading to pre-insulitis, since MCP-1 and IP-10 were not modulated by the diet or taurine. Although the addition of taurine to protein-restricted diet protected adult islets from apoptosis and NO overproduction, unnecessary supplementation could have deleterious effect on the endocrine pancreas.

0437

Role of beta cell NF-κB activation and anti-apoptotic preconditioning in Gleevec-mediated protection against diabetes in the mouse

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Background and Aims: It has recently been reported that tyrosine kinase inhibitor imatinib mesylate (Gleevec) improves Type 2 diabetes and protects against arteriosclerosis. The mechanisms that underlie this beneficial effect of Gleevec are, however, unclear. The aim of the present study was to establish whether Gleevec protects against diabetes in animal models for diabetes that are characterized by beta-cell destruction.

Materials and Methods: Gleevec was administered daily to non-obese diabetic (NOD) mice by gavage from 9 to 35 weeks of age. Gleevec was also given to NMRI mice one day before, the same day and one day after a 160 mg/kg streptozotocin injection. Development of diabetes was followed by blood glucose measurements and beta-cell mass was assessed using immunohistochemical staining for insulin and morphometric analysis. For *in vitro* experiments the insulin producing cell line betaTC-6 and human islets were used.

Results: We demonstrate that Gleevec counteracted diabetes in the streptozotocin-injected mouse and the NOD mouse, and that this was paralleled by a partial restoration of the beta-cell mass. Gleevec affected neither insulitis nor splenocyte cytokine production in NOD mice. In addition, Gleevec decreased beta-cell death *in vitro* in response to streptozotocin, proinflammatory cytokines and a nitric oxide donor. The Gleevec effect was mimicked by knock-down of c-Abl using the RNAi-technique. Gleevec probably enhanced beta-cell survival by promoting a state similar to ischemic preconditioning, as evidenced by NF-kappaB activation, increased nitric oxide and hydrogen peroxide production and depolarization of the inner mitochondrial membrane. Pharmacological inhibition

of NF-kappaB counteracted the benificial effects of Gleevec.
Conclusion: We conclude that Gleevec mediates beta-cell survival and that this could contribute to the beneficial effects observed in diabetes.
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PS 20

Pancreas and islet transplantation

0438

Impaired stimulation of insulin secretion by GLP-1 after islet auto- and allo-transplantation in humans

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Background and Aims: Intrahepatic islet auto-transplantation (AUTO) following pancreatectomy for chronic pancreatitis would be expected to be more durable than allo-transplantation (ALLO) for treating type 1 diabetes because of avoidance of immunosuppression, but the control of insulin secretion in either tranplantation has not been studied. Our aims were to study insulin action and secretion in AUTO and ALLO patients and compare results to normal (NL) subjects.
Materials and Methods: Forty-six year old ALLO female (studied pre-pancreatectomy and 1 and 3 months after transplantation) who received 550,000 islet-like clusters, 10 ALLO patients (studied 1 month after transplantation) who received on average 910,000 islet-like clusters, and 6 NL subjects. We first performed hyperinsulinemic-euglycemic clamps (80 mU/kg/min) for 2 h. Following 1 h recovery, we then raised plasma glucose levels by 5.4 mmol/l with a primed infusion of 20% glucose from 0–120 min and infused glucagon-like peptide-1 (GLP-[7–36]amide,1.5 pM/kg/min) from 60–120 min. Mean plasma insulin levels (IRI, pM) were calculated for each hour (means±SEM are shown).
Results: In both ALLO and AUTO patients peripheral tissue sensitivity to insulin was increased following transplantation. In NL subjects, hyperglycemia alone resulted in an IRI (0–60 min) of 138±24 pM that was similar to that of the AUTO patient pre-op (74 pM). ALLO patients had a similar response to hyperglycemia alone (185±30 pM), but had absent first-phase (0–10 min) response. One month after transplant, the AUTO patient showed a profound decrease overall in insulin secretion in response to hyperglycemia (36 pM) and a similarly defective first-phase insulin secretion to ALLO. By 3 months post-op, the AUTO patient had regained IRI responsiveness to hyperglycemia (90 pM) but still had absent first-phase secretion. GLP-1 infusion caused a 14-fold increase in glucose-stimulated IRI secretion in NL (IRI 60–120 min=1963± 350 pM) that was similar to the pre-op result in AUTO (1412 pM). In ALLO patients the GLP-1 augmentation of IRI was merely 3-fold (to 588 pM). The one month post-op IRI of the ALLO patient to GLP-1 was augmented only a 1.6-fold (to 57 pM) in reponse to hyperglycemia. Three months after auto-transplantation, the GLP-1 augmentation of glucose-mediated IRI had increased 3-fold (to 280 pM).
Conclusion: Beta-cell responsiveness to glucose after islet transplantation is influenced by the quality and quantity of infused islets and the necessity for immuno-suppression, and may also be influenced in both allo- and auto-transplants by the denervation status of the islet graft. First-phase insulin secretion is impaired after islet transplantation. Additionally, there appears to be a selective loss of responsiveness to GLP-1 in both auto- and allo-transplants. Three months after auto-transplantation, this deficit had lessened, and further studies will ascertain if the alterations in insulin release and responsiveness to GLP-1 persist long-term in intra-hepatic islet engraftment. The increased insulin sensitivity can be ascribed to insulinopenia following transplantation.
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0439

Long-term follow-up of simultaneous islet-kidney transplantation

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Background and Aims: Recently the beta-score was proposed as a simple clinical scoring system for islet function and diabetes control, since this integrated measure is more useful than the presence or absence of insulin independence in recipients of islet transplantation alone (ITA). We assessed beta-cell and renal function in recipients of simultaneous islet-kidney transplantation (SIK) with the beta-score and creatinine-clearance over a period of up to 6 years.

Materials and Methods: 13 consecutive patients with type 1 diabetes received SIK with a median follow-up of 3.2 years (range 1.0–6.0 years). Two patients were lost to follow-up and, therefore, excluded from analysis. The beta-score as a composite measure consists of glycated hemoglobin, stimulated C-peptide, need for insulin or oral antidabetic drugs, and fasting plasma glucose. For each of these variables 0, 1 or 2 points were assigned (maximum score=8 and a score >4 can only be achieved with endogenous insulin production).
Results: The means of the following parameters over the whole follow-up period were calculated: Daily insulin dose decreased by 54% from 0.59+0.18 U/kg BW pretransplant to 0.27+0.18 U/kg BW posttransplant. Although insulin independence in patients with>2 islet transplants decreased from 71% to 29% at the end of the follow-up period, glucose control was excellent as assessed by HbA1c and beta-score. HbA1c was 6.2 +0.4% post– vs. 8.1+1.5% pretransplant and the beta-score remained stable at 4.9+ 0.8 vs. 1.3+0.3 pretransplant, respectively. During follow-up there were no incidents of severe hypoglycemic episodes and creatinine and creatinine clearance were 136+16 mol/l and 48+5 ml/min/1.73 m^2, respectively.
Conclusion: We extend the applicability of the beta-score as a useful integrated measure of beta-cell function to recipients of SIK with a follow-up up to 6 years. Renal function and beta-score remained stable over time. Despite more advanced diabetes complications a beta-score as good as reported in ITA could be achieved after SIK.

0440

Viral transduction efficiency and gene expression in pseudo-islet aggregates with HIV-based lentiviral vectors: *in vitro* and *in vivo* analysis

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Background and Aims: Gene therapy using virus-based vectors is an appealing approach to target pancreatic islets to improve outcome in islet transplantation. Low transduction efficacy, viral-induced antigenicity and toxicity leading to increased apoptosis are limiting factors. In this study, we aimed at improving transduction efficacy with lentiviral transfer into pancreatic islets with acceptable cell loss and without cytotoxicity.
Materials and Methods: We studied transduction efficacy (scoring for green fluorescence) and viability (Hoechst/PI staining) in FACS-purified β-cells and pseudo-islet aggregates at different multiplicity of infection [(MOI) 5 and 50] and at different culture times [16 and 90 hours after infection with a replication-defective vesicular stomatitis virus (VSV-G) pseudo-typed lentiviral vector expressing enhanced green fluorescent protein (EGFP) under transcriptional control of the cytomegalovirus (CMV) promotor (LV-pWPT-CMV-EGFP)].
Results: At 90 hours after transduction, transfection efficacy in whole islets was low, resulting in modest EGFP expression in the islet rim with no EGFP positive cells in the islet core (3.1±1.4 and 11.2±4.1% EGFP positive cells, at a MOI of 5 and 50 respectively). Viability 90 hours after transfection was similar to uninfected controls (>90% living cells), regardless of viral concentrations used. In contrast to whole islets, FACS-purified primary rat β-cells had 81.0±1.0% of β-cells expressing the reporter gene 90 hours post-transduction at a MOI of 5. No viral-related cytotoxic effects were observed (92.3±1.8% viable cells at 90 hours culture, compared to 89.6±2.0% viable non-infected cells at 90 hours culture, NS). Pseudo-islet aggregates, prepared by further dissociation of islets with dispase and transduced at single cell level with LV-pWPT-CMV-EGFP before re-aggregation to islet clusters, had transduction rates of 80.0±2.8% at MOI 5 already 64 hours post-infection with maintained viability (83.5±12.0 living cells). There was, however, an important cell loss during preparation of pseudo-islet aggregates, as evidenced by a 10-fold decrease in insulin content. However, surviving cells did not show enhanced susceptibility to cytokine-induced cell death and their response to glucose was preserved. When using pseudo-islet aggregates (infected or not) for transplantation, at least 1,700,000 cells were needed to correct hyperglycemia in alloxan-induced diabetic NOD-SCID mice. Graft-bearing kidneys (containing functioning LV-pWPT-CMV-EGFP-transduced pseudo-islet aggregates) were strongly EGFP positive exclusively in the graft up to 90 days after transplantation. EGFP expression co-localized with insulin-positivity, confirming the presence of EGFP in β-cells and excluding viral spread to surrounding tissues. Moreover, lentiviral-transduced and non-transduced pseudo-islet aggregate groups had similar glucose responsiveness and immune susceptibility *in vivo*.
Conclusion: our data demonstrate that dispersion of islets prior to lentiviral transfection and re-aggregation prior to transplantation is an efficient way to introduce genes of interest into islets for transplantation purposes. However, important cell loss is the high price to pay for this improved transfection efficacy.

0441

Anti-angiogenic and immunomodulatory effects of rapamycin on pancreatic islet endothelial cells: relevance for islets engraftment after transplantation

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Background and Aims: Donor intra-islet endothelial cells contribute to islets neovascularization after tranplantation. Several factors may interfere with this process and ultimately influence islet engraftment. Rapamycin, a central immunosuppressant in islet transplantation, is a mTOR inhibitor that has been shown to inhibit cancer angiogenesis. The aim of this study was to evaluate the effects of rapamycin on the endothelium from freshly purified islets and on islet-derived endothelial cell lines.
Materials and Methods: The outgrowth of endothelial cells from freshly purified human islets and the formation of capillary structures on Matrigel were studied *in vitro* and *in vivo*. Human (hIECs) and mouse (mIECs) islet endothelial cell lines were isolated and analyzed for proliferation, apoptosis, motility and angiogenesis capacity in the presence or absence of rapamycin. In parallel, selected vascular growth factors and cell surface receptor expression were studied at gene and protein levels.
Results: Rapamycin inhibited the outgrowth of endothelial cells from freshly purified human islets and the formation of capillary-like structures *in vitro* on matrigel-coated surfaces and *in vivo* after subcateneous injection into SCID mice. Rapamycin decreased migration, proliferation and angiogenic properties of both hIECs and mIECs with appearance of apoptotic features in a dose-dependent manner. Gene-array analysis of angiogenesis-related genes on human islets revealed a decreased expression of several endothelial growth factors, CD31 (PECAM-1), alphaVbeta3 integrin and thrombin in presence of rapamycin. Western blot and FACS studies confirmed the down-regulation of angiogenic factors. On the other hand, rapamycin reduced the expression of molecules involved in immune processes such as ICAM-1 and CD40 and reduced the adhesion of T-cells to hIECs.
Conclusion: Our results suggest that rapamycin exerts dual effects on islet endothelium inducing a simultaneous inhibition of angiogenesis and a down-regulation of receptors involved in lymphocyte adhesion and activation.

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0442

Adenoviral overexpression of insulin-like growth factor II increases beta cell replication and mass in transplanted islets

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Background and Aims: In the initial days after transplantation more than half of transplanted tissue is lost. Insulin-like growth factor-II (IGF-II) is a growth promoting peptide which is able to stimulate β-cell proliferation and survival. The aim of this study was to determine whether the IGF-II overexpression in rat islets could have a beneficial effect on transplanted islets.
Materials and Methods: Islets from Lewis rats were infected ex vivo with adenovirus encoding for IGF-II (Ad-IGF-II). Streptozotocin-diabetic Lewis rats were syngeneically transplanted with 500 Ad-IGF-II infected islets (Ad-IGF-II group) or 500 uninfected islets (control group) under the kidney capsule. 500 islets are an insufficient mass to restore normoglycemia; thus, we expected that all animals would remain hyperglycemic after transplantation. Grafts were removed 3, 10 and 28 days after transplantation and beta cell replication (BrdU incorporation) and mass (morphometry) were determined.
Results: All animals showed similar hyperglycemia after transplantation ensuring comparable metabolic conditions among groups throughout the study. In Ad-IGF-II group, beta cell replication was higher than in normal pancreas (0.35±0.06%; $p<0.001$) and it was also higher than in control group on days 3 (1.48±0.07% *vs* 0.59±0.02%; $p<0.001$), 10 (1.37±0.13% *vs* 0.93±0.15%; $p<0.01$) and 28 (1.42±0.06% *vs* 0.67±0.04%; $p<0.001$). It is noteworthy that in control group beta cell replication was increased only on day 10 but not on days 3 and 28 after transplantation, despite the exposure to similar hyperglycemia in all time points. IGF-II overexpression was therefore able to overcome this initial limitation in beta cell replication (day 3), as well as that induced by long-term exposure to hyperglycemia (day 28). Beta cell mass was reduced (approx. 60%) on day 3 after transplantation in control (0.39±0.03 mg) and Ad-IGF-II group (0.49±0.09 mg). Thus, IGF-II overexpression did not prevent the dramatic beta cell loss that took place in the

initial days after transplantation. On day 10 after transplantation, beta cell mass was increased in Ad-IGF-II group (1.39±0.18 mg; $p<0.05$) and also in control islet grafts (1.20±0.09 mg; $p<0.05$) paralleling the increased beta cell replication in both groups. On day 28, beta cell mass decreased in control (0.31±0.07 mg) and Ad-IGF-II groups (0.78±0.17 mg; $p<0.05$), but it was partly preserved in IGF-II group ($p<0.05$ vs control group), in agreement with the increased beta cell replication in IGF-II group on day 28, indicating a protective effect of IGF-II overexpression on beta cell mass.

Conclusion: IGF-II overexpression in transplanted islets was able to increase beta cell replication and mass in islets grafts, and partly protected them from the deleterious effects of chronic hyperglycemia on beta cells. IGF-II may be useful to design strategies aimed to preserve beta cell mass in transplanted islets.

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0443

Successful outcome after intraperitoneal transplantation of non-PLL high G barium-alginate micro-encapsulated islets

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Background and Aims: Microencapsulation protects islets from the host immune system and thus allows for transplantation without use of immunosuppressive drugs. In type 1 diabetes, the transplanted cells need to be protected from immune rejection as well as the ongoing autoimmune reaction. Alginate is the most commonly used polymer for cell encapsulation. The properties of the alginate gel depend on the alginate composition. High guluronic acid (G) sequences are required for the alginate to form a stable and strong gel, but also make the gel more permeable than high mannuronic acid (M) gels. Traditionally, a poly-L-lysine (PLL) layer is added to the alginate core. However, PLL reduces the biocompatibility of implanted capsules by provoking an immune response and inducing a cellular reaction around the capsule. Most previous studies have been carried out on PLL capsules transplanted into animals with chemically induced diabetes. In this study, the polycation layer was omitted, and high G barium-alginate capsules transplanted into mice with spontaneous diabetes.

Materials and Methods: Balb/c or C57BL/6J islets were encapsulated in high G alginate, using $BaCl_2$ and $CaCl_2$ as crosslinkers. Thereafter, 700 encapsulated islets were transplanted into the peritoneal cavity of alloxan-diabetic C57BL/6J mice or spontaneously diabetic NOD mice. In addition, the same number of non-encapsulated islets and empty capsules were transplanted into diabetic C57BL/6J and NOD mice, respectively. Blood glucose concentrations were monitored once a week for up to 14 weeks after transplantation. Retrieved capsules were counted and visually evaluated for cellular overgrowth.

Results: Diabetic C57BL/6J mice transplanted with encapsulated syngeneic or allogeneic islets were normoglycemic for 23±7 and 80±18 days. Quite in contrast, both syngeneic and allogeneic non-encapsulated islets failed to reverse hyperglycemia. NOD mice grafted with encapsulated Balb/c and C57BL/6J islets remained normoglycemic for 67±14 and 84±10 days, respectively. The implanted empty capsules did not normalise blood glucose levels at all. In NOD mice, retrieval rates and cellular overgrowth on capsules containing islets on the one hand and empty capsules on the other did not differ.

Conclusions: As few as 700 intraperitoneally grafted encapsulated islets can reverse hyperglycemia in mice with alloxan-induced diabetes or in NOD mice. Non-encapsulated syngeneic and allogeneic islets lacked this property. This indicates that barium capsules without PLL protect the islets from immune rejection. Moreover, they also seem to serve as a matrix to support the encapsulated islet. The fact that the retrieved capsules had little or no cellular overgrowth indicates that this high G barium-alginate capsule without PLL is highly biocompatible. In a previous study we found that retrieved encapsulated islets had low overall glucose oxidation and insulin release rates. This most probably reflected a decrease in beta cell mass of each islet. Despite that, the islets still responded well to an acute glucose challenge, indicating that the function of the beta cells was well preserved. Taken together these and previous data from our laboratory support the view that high G capsules lacking a PLL-layer are superior to conventional PLL-covered alginate capsules.

0444

Mesenchymal stem cells modulate allogenic response in pancreatic islet transplantation

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Background and Aims: Pancreas transplantation is currently the gold-standard therapeutic approach for type 1 diabetic patients which consistently provides insulin-independence. Intrahepatic human islet transplantation is a therapeutic option for diabetes mellitus (type I); however, the need for a substantial amount of islets and repeated transplantations indicate impaired survival or function of islets. The main obstacles for clinical islet transplantation are the need for pancreatic islets derived from multiple donors and the adverse effects of current immunosuppressive protocols. Mesenchymal stem cells (MSCs) are multipotent adult stem cells capable of differentiate in various cell lines like ostocytes, adipocytes, chondrocytes but also in neuronal and endothelial phenotypes. The MSCs escape from the immune system by suppression of lymphocyte proliferation and modulation of allogenic response *in vitro*. In vivo they prolong skin allograft survival and may decrease graft-versus-host disease after hematopoietic stem cell transplant. Therefore, we investigated the immunomodulatory effects of MSCs in an experimental model of marginal-mass islet transplantation.
Materials and Methods: Islets and bone marrow-derived MSCs were isolated from inbred Sprague-Dawley rats. Streptozotocin-induced diabetic Wistar rats underwent intraportal transplantation (700 IE) and simultaneous portal or systemic MSCs infusion ($2{,}5\times10^4$, $7{,}5\times10^4$, 15×10^4/kg monodose). Blood glucose levels and insulin production (RIA) were continuously monitored, islet survival was followed by magnetic resonance imaging (MRI) and liver histology was assessed at 7th post-transplant day.
Results: Portal infusion of $2{,}5\times10^4$ MSCs does not result in a significant enhancement in glycemic control, only a faster fall (2nd post-transplant day versus 4th post-transplant day) in the glycemic level was observed. Portal infusion of a higher number ($>2{,}5\times10^4$) of MSCs causes serious thrombotic events in the liver also in presence of anticoagulative therapy. The systemic low dose ($2{,}5\times10^4$ and $7{,}5\times10^4$) infusion of MSCs does not improve allogenic islet function but 15×10^4/kg MSC increases significantly the normoglycemic period (5 days) after marginal mass islet transplant which is not able to normalize iperglycemia by itself. The histological analysis showed a lower lymphocyte infiltration around intrahepatic islet grafts in the presence of MSCs in both type of infusions.
Conclusion: Systemic high doses of MSCs improve glycemic control in our animal model and reduce lymphocyte infiltration in acute rejection. These data suggests that MSC mantain their *in vitro* properties of suppressing lymphocyte activation and proliferation also *in vivo*. The above described results of MSC need more investigation to define whether the observed effect is an allospecific process or part of a general antiinflammatory effect.
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0445

Effects of immunosuppressive drugs on human islet neogenesis *in vitro*

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Background and Aims: Immunosuppressive drugs may have deleterious effects on transplanted islets. In vitro studies have shown that these drugs have negative effects on beta-cell viability and insulin synthesis and secretion. In previous studies, we reported the generation of endocrine cells from human adult pancreatic ductal cell cultures. Assuming that neogenesis contributes to the long-term function of islet grafts, it is important to study the effects of immunosuppressive drugs on precursor cell proliferation and differentiation. We have now examined the effects of clinically used doses of immunosuppressive drugs on these processes *in vitro*.
Materials and Methods: Freshly isolated human pancreatic cells from 8 donors were used in this study. The culture consists of an expansion phase in serum-containing medium followed by Matrigel overlay and a differentiation phase in serum-free conditions. More than 90% of the proliferating cells were CK19-positive ductal cells. Immunosuppressive drugs, including sirolimus (10 ng/ml), tacrolimus (5 ng/ml), mycophenolate mofetil (MMF, 20 μg/ml), daclizumab (10 μg/ml) and their combinations were tested in parallel culture wells through either the expansion phase (5–7 days) or the entire culture period (4–5 weeks).
Results: MMF, either alone or in combination with sirolimus or tacrolimus, severely hampered duct-cell proliferation by 8-fold during the expansion period, and significantly reduced the total DNA content by about 40% after 5-week culture. After 4–5 week exposure to different drugs, only sirolimus and daclizumab showed no adverse effects on insulin content, whereas significant reductions of 30–60% in insulin content were seen in all other experimental groups. Only tacrolimus decreased the insulin content per DNA, as well as the proportion of insulin-positive cells by immunocytochemistry.
Conclusion: Based on these *in vitro* results, MMF has a potent inhibitory effect on human islet neogenesis primarily through an antiproliferative effect on the precursors, whereas tacrolimus mainly affects beta-cell differentiation. Sirolimus and daclizumab have no adverse effects on these parameters. The immunosuppressive protocol may be an important determinant of long-term clinical islet graft function.

0446

Characterisation of a rat model of diabetes induced by prolonged treatment with cyclosporin A and FK-506

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Background and Aims: The immunosuppressants cyclosporin A (CsA) and tacrolimus (FK-506) may induce "postransplant diabetes" with a relatively high prevalence in organ transplanted patients. We have developed a rat model of this type of diabetes with the aim of studying the diabetogenic mechanism of action of the two immunosuppressants.
Materials and Methods: Groups of 6 rats were injected (i. p.) twice a day 0.15 ml of either saline (control), CsA (7.5 mg/Kg BW), or FK-506 (0.1 mg/Kg BW). Body weight and glycaemia were regularly recorded. Islets from either group of treated rats were isolated by collagenase digestion of the pancreas. Islet insulin was measured radioimmunologically and DNA fluorimetrically, after reaction with diaminobenzaldehyde.
Results: Rats (groups of 6) treated with CsA and FK-506 developed hyperglycaemia, as compared with control rats (175.5±12.4 and 159.8±8.9, respectively, vs. 100.8±2.7 mg/dl; $p<0.0002$ and $p<0.0001$), after 7 days and it persisted until day 17 (204.5±28.1 and 153.2±8.4, vs. 97.3±2.1 mg/dl; $p<0.004$ and $p<0.0001$) in which the immunosuppressant treatment was withdrawn. Ten days later, the glycaemia started to return towards normality (112.0±5.5 and 103.8±2.9, for CsA and FK-506, respectively, vs. 92.2±1.3 mg/dl in the controls; $p<0.006$ and $p<0.005$). The pancreatic insulin content was reduced by either CsA and FK-506 after 17 days of treatment (40.0±3.6 and 54.09±2.2, respectively, vs. 104.6±8.3 ng/mg pancreas; $p<0.001$ in both cases) but these differences were completely abolished 14 days after the withdrawal of treatment (111.0±9.4 and 104.3±10.8, vs. 120.1±7.2 ng/mg pancreas). The immunossupressants retarded significantly (FK-506) or completely suppressed (CsA) the increase of body weight during the treatment period. Islets isolated from rats treated for three days with CsA or FK-506 did not show any modification of their insulin and DNA content, nor of their secretory response to 20 mM glucose. However, *in vivo* treatment with CsA for 5 days strongly decreased the islet insulin content (75.9±8.6, n=6, vs. 231.2±9.9 ng/islet, n=6; $p<0.001$) as well as the DNA content (16.8±1.4,n=4, vs. 24.2±1.2 ng/islet,n=5;$p<0.005$) and the secretory response to 20 mM glucose (10.2±1.6,n=6, vs. 24.0±1.5 pg/islet x min,n=6;$p<0.001$). By contrast, 5 days of treatment with FK-506 did modify neither the DNA content nor the secretory response to 20 mM glucose of islets but reduced their insulin content (143.2±12.4,n=6, vs. 231.2±9.9 ng/islet, n=6;$p<0.001$). After 7 days of treatment with FK-506, the islet content of insulin was similarly suppressed within 50% (128.7±12.7,n=6,vs.274.1±31.5 ng/islet,n=6;$p<0.003$) but the islet insulin response to 20 mM glucose was now diminished by more than 75% (8.0±2.2, n=6, vs. 33.9±6.0 pg/islet x min, n=6;$p<0.01$).
Conclusion: Prolonged treatment of rats with CsA and FK-506 induces hyperglycaemia and reduced insulin content of the islets, mimicking the post-transplant diabetes observed in patients. This "diabetic" state is easily reversible shortly after immunosuppressant withdrawal. The observed decrease of the islet insulin content is probably responsible of the diminished insulin response to glucose. We are currently investigating whether the reduction of islet insulin content might be attributed to a decrease of rat insulin genes' transcription and whether apoptosis is also contributing with a decreased β-cell mass.
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PS 21

Immunopathogenesis of type 1 diabetes

0447

The frequency and function of CD4+CD25+T regulatory cells in the diabetic initiation and progression of nonobese diabetic mice

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Background and Aims: Type 1 diabetes is an autoimmune disease caused by selective destruction of islet beta cells in the pancreas mediated by pathogenic T cells. Some studies have found $CD4^+CD25^+$ T regulatory cells($CD4^+CD25^+$ Treg cells) plays an important role in preventing the induction of several autoimmune diseases. Our studies investigated the frequency and function of $CD4^+CD25^+$ Treg cells in the diabetic initiation and progression of nonobose diabetic mice.
Materials and Methods: Early-preclinical-stages(4W) female NOD mice were kept under specific pathogen-free conditions. The number of $CD4^+CD25^+$ Treg cells was observed per two weeks from 4 to 32 weeks of age. Cells from peripheral blood were stained with TC-CD3,FITC-CD4 and PE-CD25 antibodys. Production of intracellular cytokine IL-4 and IFN-γ were measured with flow cytometry. To assess their funtion, $CD4^+CD25^+$ Treg cells were cocultured with $CD4^+CD25^-$ T cells at 32 weeks of age. The expressions of Foxp3 were measured by RT–PCR. Pancreata were prepared for the histology. Insulitis severity were scored by hematoxylin and eosin.
Results: 1. The frequencies of $CD4^+CD25^+$ Treg cells of female NOD mice were progressively lower from 4 to 32 wekks of age($p<0.05$).

2. The numbers of $CD4^+CD25^+$ Treg cells of diabetic NOD mice of 32-week-old was significantly lower than nondiabetic ones(p=0.01), and with increased secretion of IFN-γ and decreased IL-4 production.
3. The suppressor function of the $CD4^+CD25^+$ Treg cells *in vitro* cocultures was reduced which was similar between diabetic and nondiabetic mice.
4. The insulitis of diabetic mice with≥50% of infiltration of the islets at 32 weeks of age was more severe than nondiabetic ones.
Conclusion: 1. The progression to overt diabtes correlates with the age-dependent decrease in the number of and deficiency in the function of the $CD4^+CD25^+$ Treg cells, which cannot maintain the tolerance to islet autoantigens effectively. 2. This study give a direct and strong evidence to support that $CD4^+CD25^+$ Treg cells contribute to the development of autoimmune diabetes. It maybe give us a new method to screen and treat the type 1 diabetes by Treg therapy.
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0448

Insulitis severity and pancreatic expression of pathogenic lymphocytes markers CXCR3 and its chemokine ligands are reduced in probiotic treated NOD mice and is inversely correlated with IL-10 production

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Background and Aims: In a previous study we have demonstrated that, in the NOD mouse, oral probiotic administration, via an immune-modulation of the gut associated lymphoid tissue (GALT) prevented autoimmune diabetes, reduced insulitis severity, and induced an upregulation of IL-10 production by lymphocytes in Peyer's Patches, spleen and pancreas. Recent studies focused on the role of proinflammatory chemokines in the pathogenesis of autoimmune disease. In particular in type 1 diabetes, it has been demonstrated the pathogenetic role of $CXCR3^+$ autoreactive lymphocytes, recruited in the pancreatic islet by IP-10/CXCL10 and MIG/CXCL9, secreted by islet cells. We aimed to further characterize the mechanisms of diabetes prevention induced by VSL#3 probiotic compound administration.
Materials and Methods: Four weeks old, female NOD mice (n=18) were randomly divided into 2 groups to receive orally VSL#3 or PBS, 3 times/week, for a total of 8 weeks. At 12 weeks of age the animals were sacrificed and pancreata were collected. IL-10, IFN-γ, TNF-α, CXCL9, CXCL10 and CXCR3 mRNA expression was evaluated by Real Time PCR. Histological and immuno-histochemical studies were performed to characterize the insulitis.
Results: In the VSL#3 treated mice, a significant reduction of the insulitis score ($p<0.03$) has been observed, together with an up-regulation of IL-10 expression ($p<0.0005$), and a reduced expression of proinflammatory cytokines, IFN-γ ($p<0.03$) and TNF-α ($p<0.05$), and of proinflammatory chemokines, IP-10 ($p<0.0005$) and MIG ($p<0.0005$) and of their receptor, CXCR3 ($p<0.0005$). IP-10 was expressed by islet cells, as shown by immuno-histochemical staining. Confirming the protective role of IL-10, IL-10 pancreatic expression was inversely correlated with both insulitis severity ($p<0.02$), as well as with the expression of the lymphocytes marker, CXCR3 ($p<0.0005$). As expected, a positive correlation existed between the pancreatic expression of the receptor CXCR3 and its ligands IP-10 ($p<0.0001$) and MIG ($p<0.0001$).
Conclusions: This study indicates that diabetes prevention induced by oral VSL#3 administration is associated with local reduction of β-cell expression of proinflammatory chemokines and, as a consequence, reduced islet infiltration by pathogenic $CXCR3^+$ lymphocytes as well as increased production of IL-10.
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0449

Gimap/GTPase family is highly expressed in the lymphoid organs of the Bio-Breeding rat

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Positional cloning of the lymphopenia (lyp) gene in diabetes prone (DP) Bio-breeding (BB) rats revealed a frameshift mutation in the Gimap5 (GTPase of immunity associated protein)(formerly called Ian5) a member of at least six other related genes comprising the Gimap family expanded in 150 kb on rat chromosome 4q24. To characterize this gene family and understand how it may function in lymphopenia and diabetes development, we cloned and sequenced each of the seven rat Gimap genes from our congenic BBDR.lyp line, both from the+/+and the lyp/lyp rats. Several amino acid differences in this gene family were found between+/+and lyp/lyp rats. In addition to the mutation in lyp/lyp rats in Gimap5 that causes a frameshift and premature truncation of the protein, Gimap1 from lyp/lyp rats has a threonine at position 251 instead methionine, and Gimap4 from lyp/lyp rats has a frameshift of three amino acids from the C terminus that results in a protein that is 18 amino acids longer than in the+/+rats. RNA expression analyses by quantitative real-

time PCR, from each of these genes revealed unexpectedly, that in spleen and mesenteric lymph nodes the T cell subsets in each of the seven Gimap genes were reduced in the lyp/lyp compared with+/+rats, even though only the mutation in Gimap5 is linked to lymphopenia and diabetes. To characterize further the specific cell types that show this coordinate regulation, we analyzed the Gimap gene expression in the thymus, spleen and mesenteric lymph nodes in sorted T CD4 +, CD8+cell subsets and the T (CD45+CD45RA−) and the B (CD45+CD45RA+) cell subsets in peripheral lymphoid organs. The entire Gimap family was found to be down-regulated in the periphery of these cell subsets of the lyp/lyp compared with+/+rats. Our data suggest that RNA Gimap gene family expression is controlled by the absence of the Gimap5 protein in the BB rat.

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0450

Introgression of F344 rat genomic DNA on BB rat chromosome 4 generates diabetes-resistant lymphopenic BB rats

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Background and Aims: Failure to express the *Gimap5* protein is associated with lymphopenia (*lyp*) and linked to spontaneous diabetes in diabetes prone (DP) BB rats. *Gimap5* is a member of seven related genes located within 150 Kb on rat chromosome 4. All parental congenic DR.*lyp/lyp* BB rats develop diabetes between 46–81 days of age (mean 61) while DR.*lyp*/+and+/+rats are non-lymphopenic and diabetes resistant (DR). In an intercross between F1(DR.*lyp/lyp* x F344) rats we identified a rat with a recombination event on chromosome 4, allowing us to fix 33Mb of F344 between D4Rat253 and D4Rhw6 in the congenic DR.*lyp/lyp* rats. *Gimap1* and *Gimap5* were the only members of the *Gimap* family remaining *lyp/lyp*.

Our aim was to test whether the F344 introgression proximal to *Gimap5* resulted in 1) no effect on diabetes development or 2) protection from diabetes. The first outcome would underscore lymphopenia and the *Gimap5* mutation as a diabetogenic factor. The alternative outcome would identify a diabetogenic factor different from lymphopenia.

Materials and Methods: We secured introgression of the F344 recombination onto the congenic DR.*lyp* rat through a series of marker assisted crosses, intercrosses and backcrosses.

Results: Offspring homozygous F344 (f/f) for the recombination were lymphopenic (85/85; 100%) but did not develop diabetes (0/85). During rescue of the recombination, (102/163; 63%) rats heterozygous (b/f) for the recombination developed diabetes between 52–222 days of age (mean 88). *In silico* analysis confirms that the previously reported *Iddm4* locus is present within the 33Mb F344 DNA fragment.

Conclusion: This data demonstrates that introgression of F344 DNA proximal to the mutated *Gimap5* gene renders the rat diabetes resistant despite being lymphopenic. Our data suggest that spontaneous diabetes in the BB rat is controlled, in part, by a diabetogenic factor(s) unrelated to the *Gimap5* mutation on rat chromosome 4. The molecular identification of this genetic factor(s) should prove critical to disclose the mechanisms by which diabetes develops in the BB rat.

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0451

The mutation leading to T1DM and altered T-cell content in LEW.1AR1-*iddm* rats is located at the telomeric end of chromosome 1

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Background and Aims: The LEW.1AR1-*iddm* rat is an animal model of human Type 1 diabetes mellitus (T1DM), arose through a spontaneous mutation within the inbred strain LEW.1AR1. Genetic analyses using a (BN x LEW.1AR1-*iddm*) x LEW.1AR1-*iddm* backcross population revealed three diabetes susceptibility loci (*Iddm1*, *8*, *9*). Additionally, we observed an altered T-cell content in peripheral blood lymphocytes (PBL) within the LEW.1AR1-*iddm* population in comparison to that of the coisogenic background strain. The mode of inheritance of this deviant phenotype is autosomal recessive. It was the aim of the study, (a) to investigate a different backcross population [(PAR x LEW.1AR1-*iddm*) x LEW.1AR1-*iddm*] to narrow the susceptibility regions and (b) to identify the gene locus responsible for the altered T-cell content.

Materials and Methods: T-cell content in PBLs (peripheral blood lymphocytes) was analysed using FACS™ analyses with monoclonal antibodies. 61 microsatellite markers were used for linkage analyses of 130 (PAR x LEW.1AR1-*iddm*) x LEW.1AR1-*iddm* backcross animals.

Results: Flow cytometry showed a significantly lower $CD3^+$ T-cell content in PBLs of diabetic PAR backcross animals in comparison to the LEW.1AR1 background strain (39.6±1.28

vs. 68.9±1.04). This result approves the previous findings within the BN backcross. Linkage analyses of the (PAR x LEW.1AR1-*iddm*) x LEW.1AR1-*iddm* backcross population showed in opposite to the BN backcross (*Iddm1*, *8*, *9*) only two susceptibility loci, one on chromosome (RNO) 1 (*Iddm8*) and one in the MHC region on RNO20 (*Iddm1*) responsible for the diabetes disease. *Iddm9* was missing in the PAR backcross. In accordance with previous findings, the locus responsible for alteration of the T-cell content within the PAR backcross population was also located at the distal end of RNO1 in immediate vicinity to *Iddm8* (LOD>6) flanked by *D1Ztm19* and *D1Ztm17* (213Mb).

Conclusion: The findings of the linkage analyses show that the mutation of the LEW.1AR1-*iddm* rat maps within the *Iddm8* region at the telomeric end of RNO1. Additionally, this mutation is also responsible for alterations in the T-cell content of PBLs, which may also contribute to diabetes development. Interestingly, the centromeric part of *Iddm8* (*RNO1q42-43, D1Rat295 - D1Rat496)* is homologues to *HSA11q13* containing *IDDM4*. Thus, alterations of peripheral T-cell content are linked to the *Iddm8* locus and may allow the elucidation of the network of genetic and immunological factors responsible for disease manifestation in this T1DM model.

0452

Islet extracellular matrix stimulates inflammatory mediator secretion by macrophages

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Background and Aims: Macrophages play a critical role in the inflammatory responses occurring after islet transplantation as well as in the onset of type I diabetes. Whether extracellular matrix (ECM) in islets affects inflammatory processes mediated by macrophages is still unknown. Laminin-5 gamma 2 subunit was detected by western-blotting and immunochemistry in islets. The aims of this study are i) to investigate whether laminin-5 (LN-5) is able to activate macrophages and ii) to elucidate the signaling pathways involved in this process.

Material and Methods: As source of LN-5, we used a matrix produced by a rat bladder carcinoma cell line that also contains fibronectin (804G matrix). Rat peritoneal macrophages were harvested after intraperitoneal injection of soluble starch and seeded either on non adherent plastic, collagen, fibronectin, 804G matrix or fibronectin-depleted 804G matrix. After a 24 h-incubation in presence or absence of 1 microg/ml phorbol myristate acetate (PMA), we measured by ELISA TNF-alpha, IL-6 and nitrite (NO2-) secreted by macrophages. Activation of p38 MAPK, ERK1/2 MAPK, and FAK was analysed by western-blotting.

Results: We confirmed by immunofluorescence and western blotting that laminin-5 gamma 2 subunit is expressed in islets and is localized in alpha cells. In absence of PMA, macrophages cultured on 804G showed an increased secretion of TNF-alpha and NO2- compared to plastic, collagen and fibronectin. In presence of PMA, 804G matrix potentiated the secretion of all inflammatory mediators studied compared to plastic, collagen and fibronectin. Similar effects on TNF-alpha, IL-6 and NO2- secretion were observed using fibronectin-depleted 804G matrix. After 30 min-incubation in the absence or presence of PMA, macrophages attached on 804G matrix showed an increased phosphorylation of p38 MAPK and ERK1/2 MAPK when compared to non adherent plastic. By contrast, phosphorylation of FAK was reduced under the same conditions.

Conclusion: LN-5 rich 804G matrix potentiated secretion of inflammatory mediators by peritoneal macrophages. These effects are likely to be mediated by activation of p38 MAPK and ERK1/2 MAPK.

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PS 22

Immunology and prediction of type 1 diabetes

0453

Type 1 diabetes children without islet autoantibodies at the time of clinical diagnosis are more likely to have islet autoantibodies at birth

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Background and Aims: A recent study suggested that fetal exposure to islet autoantibodies in children born to mothers with type 1 diabetes may be protective against future islet autoimmunity and diabetes. The possible induction of immune tolerance to islet autoantigen may reflect that islet autoimmunity is initiated already *in utero*.

The aim of this study was to investigate islet cell autoantibodies in an unselected population of children with type 1 diabetes, born to non-diabetic mothers, by comparing autoantibodies in their cord blood serum samples with a sample taken at the time of clinical diagnosis.

Materials and Methods: Serum samples at birth and at the time of diagnosis were available from 141 children who developed type 1 diabetes between 1.3–18.8 years of age (median 9.0 years; male/female ratio 83/58). Children born

to mothers with type 1 diabetes or any other form of diabetes were excluded from the study. The samples were tested for autoantibodies against glutamic acid decarboxylase (GAD65Ab), islet-associated antigen 2 (IA-2Ab), insulin (IAA) by radiobinding assays as well as to islet cell antibodies (ICA) by indirect immunofluorescence

Results: The frequency of islets autoantibodies in the cord blood was 11% (15/141), (ICA 4%, GAD65Ab 6%, IA2-Ab 1% and IAA 1%) compared to 91% (129/141) (ICA 76%, GAD65Ab 61%, IA2-Ab 69% and IAA 45%) at diagnosis. The children who presented fewer islet autoantibodies (Ab′s) at diagnosis were more likely to have had autoantibodies in their cord blood sample. Of children with no Ab′s at diagnosis 25% had autoantibodies in cord blood, 1 Ab 21%, 2 Ab's 17% and >3 Ab's 5% ($p<0.01$) demonstrating a significant inverse relationship (trend, $p<0.001$). Cord blood autoantibodies were most strongly associated with absence of ICA at diagnosis (OR 6.06 (95%CI was 1.97–18.60) $p=0.0016$) even after adjusting for other islet autoantibodies at diagnosis (OR 4.65 (1.23–17.5).

Conclusion: Our data in type 1 diabetes children born to non-diabetic mothers support the notion that cord blood islet autoimmunity may induce immunological tolerance and explain why some type 1 diabetes children are islet autoantibody negative at the time of clinical diagnosis.

0454

Autoantibodies specific to conformational epitopes of the 65 kDa isoform of glutamate decarboxylase (GAD65) identify different types of autoimmune diabetes

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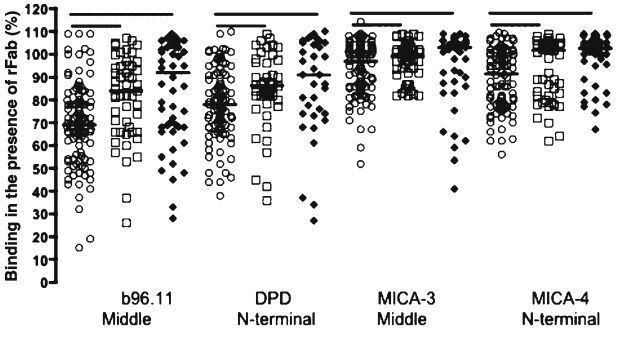
Background and Aims: Autoimmune diabetes presents itself in different subtypes. Classical type 1 diabetes (T1D) is characterized by young age at onset and rapid progression, while patients with latent autoimmune diabetes in adults (LADA) present a slow onset form of autoimmune diabetes, where patients are initially insulin-independent and often diagnosed with type 2 diabetes, before developing insulin dependency. The autoimmune reaction in both subtypes is reflected in the presence of circulating autoantibodies directed to GAD65 (GAD65Ab). We investigated whether the underlying autoimmune reactions in these subtypes differ.

Material and Methods: GAD65Ab-positive samples from Japanese T1D patients (n=111), and from LADA patients who were still insulin-independent (n=86), or had already progressed to insulin-dependency (slow onset) (n=58) were analyzed for their GAD65Ab epitope specificity using seven recombinant Fab. These recombinant Fab were derived from human GAD65-specific monoclonal antibodies that recognize different epitopes of the molecule.

Results: We identified significant differences in the GAD65Ab epitope specificities between the three groups. Overall, the T1D patients recognized more epitopes (median=3) than both slow onset (median=2) or LADA patients (median=2), (P=0.0003 and 0.0005, respectively). In particular, binding to a middle epitope was significantly better recognized by GAD65Ab in T1D patients than in the slow onset ($p=0.0002$) or in the LADA patients ($p<0.0001$). This preferred binding was also observed for two N-terminal epitopes that were significantly better recognized in T1D patients than in slow onset (P=0.009 and 0.005), or LADA patients (P=0.01 and <0.0001). The same trend (T1D>slow onset>LADA) was observed for three of the remaining four epitopes, however the differences did not reach significance. While for six of the seven epitopes the slow onset patients recognized the epitope better than the LADA patients, these differences did not reach significance.

Conclusion: The autoimmune reaction in LADA patients differs from that in T1D patients. This is reflected in their GAD65Ab epitope specificities. It will be of interest to investigate whether the epitope pattern in the LADA patients change as they progress towards insulin dependency.



Samples from T1D patients (circles), slow onset (squares) and LADA patients (diamonds), were incubated with the respective recombinant Fab and radiolabeled GAD65. Binding is reported as percentage of uncompeted binding (set as 100%). Median are indicated. Significance in binding is indicated by bars.

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0455

Comparison of islet cell autoimmunity in Japanese and Caucasian type 1 diabetes patients: Autoantibodies to the 65 kDa isoform of glutamate decarboxylase recognize similar epitopes

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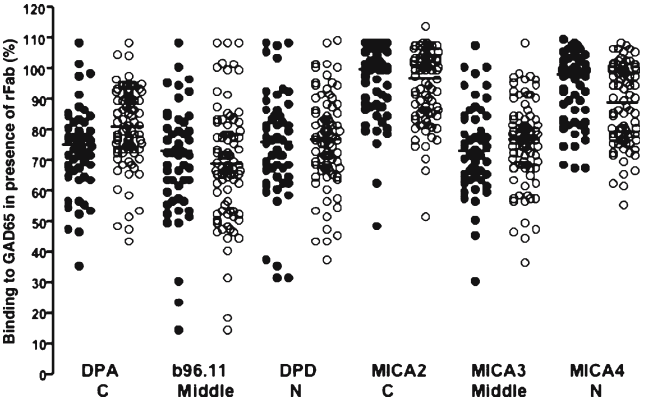
Background and Aims: The incidence of Type 1 diabetes (T1D) in the Japanese population is remarkably lower as compared with that in Caucasian populations. In both populations autoantibodies to the 65 kDa isoform of glutamate decarboxylase (GAD65), insulin, and IA-2 are prevalent at onset of disease. However, the genetic susceptibility conferred by HLA class II alleles differs significantly between the two populations. The HLA class II genes which confer the highest risk in Caucasians (DRB1*0301-DQB1*0201 and DRB1*0401-DQB1*0302), are rare in the Japanese population and instead the DRB1*0405-DQA1*0302-DQB1*0401, DRB1*0901-DQA1*0302-DQB1*0303 and DRB1*0802-DQB1*0302 emerge as the major susceptible haplotypes of T1D.
We compared the autoimmune islet cell reaction reflected in GAD65Ab epitope specificities in Japanese and Caucasian T1D patients.
Material and Methods: Japanese (n=111) and Caucasian (n=50) GAD65Ab-positive T1D patients were analyzed for their GAD65Ab epitope specificity. The sera were analyzed in a competitive radiobinding assay using six recombinant Fab (rFab) derived from GAD65-specific human monoclonal antibodies. Binding to GAD65Ab epitopes in both groups were compared using the nonparametric Mann Whitney U test. Binding to GAD65Ab epitopes was also tested for correlations with the HLA class II alleles.
Results: The overall GAD65Ab epitope pattern of Japanese T1D patients was remarkably similar to that observed in Caucasian T1D patients. Patients in both populations showed a strong binding to conformational epitopes located both in the middle and the N-terminus of GAD65, while a significantly lower reaction to a linear C-terminal epitope was observed for both populations. In the Japanese T1D patient groups we observed a significant correlation between the binding to a disease-specific epitope located in the middle of the molecule and the high risk HLA class II allele DRB1*0802-DQB1*0302 (p=0.0008), while binding to this epitope in the Caucasian T1D patients is associated with DRB1*0401/DQB1*0302.
Conclusions: While T1D susceptibility in Caucasian and Japanese populations is associated with different HLA class II alleles, we found that the GAD65Ab epitope specificity between the two populations is remarkably similar. The strong association between a GAD65Ab epitope specificity that was identified by us earlier to be disease-specific and a high risk HLA class II alleles in both the Japanese and Caucasian populations leads to the speculation that high risk HLA class II alleles may shape GAD65Ab epitope specificities and lead to the generation of disease-specific autoantibodies.



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0456

Detection of islet cellular autoimmunity in Chinese subjects initially diagnosed as idiopathic type 1 diabetes
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Background and Aims: Idiopathic type 1 diabetes(Type 1B) was defined as islet-associated autoantibody-negativity together with unknown etiologies by ADA criteria. There was obvious heterogeneity in Type 1B patients, some of the Chinese patients originally thought to have Type 1B was detected to be autoantibody positive to SOX13 or carboxypeptidase(CPH) and genetic mutation, these patients may have a specific aetiology and should not be classified as Type 1B diabetes. In this study, we tried to demonstrate the existence of islet-associated antigen-specific T cells in Type 1B patients and abnormal T cell-mediated immunity.
Materials and Methods: Peripheral blood samples were obtained from 23 classical type 1diabetes(Type 1A) patients, 29 Type 1B patients, 28 Type 2 diabetes(T2DM) and 16 healthy control subjects(Control). The diagnoses of Type 1B, Type 1A diabetes and T2DM were made based on the ADA criteria for diabetes. Islet-associated autoantibodies were determined by radioligand assay. Human peripheral blood mononuclear cells were isolated by Ficoll-Hypaque density gradients, enzyme linked immune spot (ELISPOT) assay was performed to measure the number of spots-forming (median, 75%CI). One spot-forming represents a GAD65-reactive IFN-γ -secreting T (IFN-γ-T) cell.
Results: The IFN-γ-spots number stimulated with GAD65 were 12(10.3~20.9)(median and 95%CI) in Type 1A group, 3.5(3.0~5.7) in Type 1B group, 2.0(1.3~4.0) in T2DM group

and 1.0(0.3~1.8) in Control group, respectively. The frequency of spots significantly increased in Type 1B group compared to Control group(P<0.05), and there were significantly more spots number in Type 1A group than Type 1B or Control group(both P<0.01), and no significant difference was observed between T2DM and Control group concerning the spot number(P>0.05). A positive response was scored when the spot number≥mean+2SD of Control subjects. In case of Type 1B patients 14 of 29(48.3%)had positive response to GAD65, and 18 of 23(78.3%)Type 1A patients and 1 of 28 (3.6%)T2DM patient showed positive response. No subjects (0%) positive for IFN-γ-spot were observed in Control group. It implied that approximately half(48.3%)of the so-called "Type 1B" patients and very few (3.6%)of T2DM patients were positive for GAD65-reactive IFN-γ-T cells. Whereas the number of IFN-γ-spots reactive to phytohemagglutinin (PHA) did not differ among the four groups(data not shown).

Conclusion: GAD65-reactive IFN-γ-T cells are found in some patients originally diagnosed as Type 1B with autoantibody-negative. This suggests, similar to Type 1A patients, that abnormal T cell-mediated immunity involved in the underlying aetiology and pathogenesis is also present in some Chinese patients originally diagnosed as Type 1B diabetes. More rigorous screening for these conditions is needed before classifying subjects as having Type 1B diabetes, and IFN-γ-T cells reaction against GAD65 detected by ELISPOT may contribute to the diagnosis of "autoimmune diabetes mellitus".

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0457

Markers associated with T-regulatory cells are induced at diagnosis of type 1 diabetes

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Background: Type 1 diabetes (T1D) is an autoimmune disease associated with a defective regulation of the immune system and imbalance of T-helper (Th) cells. T-regulatory (T-reg) cells are believed to control T cell reactivity and to maintain self-tolerance of the immune system. We hypothesise that the observed imbalance of subgroups of Th cells can be due to a dysregulation of T-reg cells in high-risk and T1D children.

Methods: Peripheral blood mononuclear cells (PBMC) were collected from T1D children four days post-diagnosis, from children with 35% risk of developing T1D within five years (ICA>=20 IJDF-U) and from healthy children carrying the HLA-risk haplotype DQB1*0302 or DQB1*0201 and DQA1*0501. Expression of genes associated with T-reg cells (FOXP3, CTLA-4, sCTLA-4 and TGF-beta mRNA) were detected together with endogenous rRNA by multiplex real-time RT-PCR. Secretion of TGF-beta, IL-10 and IFN-gamma were detected by protein micro array and IL-4 by sensitive ELISPOT technique from PBMC stimulated with glutamic acid decarboxylase (GAD65) and phytohaemagglutinin (PHA).

Results: Spontaneous mRNA expression of the T-reg associated genes FOXP3 (p<0.05), CTLA-4 (p<0.05) and TGF-beta (p<0.05) as well as spontaneous secretion of TGF-beta (p=0.05) and IL-10 (p=0.06) was more pronounced in newly diagnosed T1D children compared to high-risk children. Higher spontaneous mRNA expression of FOXP3 (p=0.01) and CTLA-4 (p<0.01) as well as secretion of TGF-beta (p<0.05) was also observed in T1D compared to healthy children.

Secretion of TGF-beta, after *in vitro* stimulation with GAD65, was higher in T1D children compared to both high-risk (p<0.05) and healthy (p<0.01) children. Further, expression of sCTLA-4 (p<0.01) and TGF-beta (p=0.01) mRNA, induced by stimulation with PHA, was higher in T1D compared to high-risk children. PHA-induced mRNA expression of sCTLA-4 (p<0.01), TGF-beta (p=0.01) and FOXP3 (p<0.05) as well as secretion of IL-10 (p<0.05) was found to be low in high-risk compared to healthy children. In contrast, spontaneous secretion of IFN-gamma was less pronounced in newly diagnosed T1D children than in high-risk children (p<0.05) and secretion of IFN-gamma was inversely correlated to IL-10 secretion in high-risk children ($r=-0.91$, p<0.05).

Conclusion: Increased expression and secretion of markers associated with T-reg cells was more pronounced in newly diagnosed T1D children than in high-risk and healthy children. These results suggest a disturbed interaction of T-cells with regulatory features to suppress the destruction of insulin-producing beta-cells mediated e g by Th1-like cells.

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0458

Several novel characteristics of Treg cells in newly-diagnosed type 1 diabetics and controls: preliminary results

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Background and Aims: One of the immunocyte populations that are pivotal to tolerance and autoimmunity is that of $CD4^+$ Treg (regulatory cells). Our aim was to delineate the characteristics of Treg cells in newly-diagnosed type 1 diabetics and controls, especially in view of conflicting results in the literature regarding certain cell surface markers of these cells.
Materials and Methods: Peripheral blood from six newly-diagnosed type 1 diabetes patients (ages 1.5 to 35, mean 16) and ten controls (ages 25–45, mean 28) was analysed via triple colour flow cytometry for cell surface and intracellular characteristics of the Treg ($CD4^{hi}$), $CD4^+CD25^+$ and $CD4^+CD25^-$ populations. The markers analysed for were CD28, CD45RO, CD95 (Fas), CD122 (IL-2Rβ), CD152 (CTLA-4) (cell surface and intracellular), CD220 (InsR), HLA-DR, HLA-DQ, TGFβ and TGFβRII. The cut-off line between $CD25^{hi}$ and $CD25^+$ cells was either obvious from the CD4-CD25 dot plot, or was decided based on whether the highest expressing population in the latter group conformed to the bulk characteristics of that population or to those of the former population.
Results: Newly-diagnosed type 1 diabetics had a significantly lower percent of Treg compared to normal controls (1.07+/− 0.28 vs. 2.27+/−0.75). All markers tested for were found on the membrane of Tregs from type 1 diabetics as well as normals. However, several of the markers were in a significantly different percent of Tregs from the normal group compared to the same cell type from newly-diagnosed type 1 diabetics (mean+/− SD): CD122 (40.5+/− 20.5 vs. 69.5+/−31.35), CD152 (87.2+/−14.9 vs. 78.2+/− 21.3), CD220 (82.8+/− 15.4 vs. 63.0+/− 16.5), TGFβ (88.7+/− 6.4 vs. 72.3+/− 14.3), HLA-DR (59.4+/− 15.4 vs. 74+/− 35), HLA-DQ (34.5+/− 24.3 vs. 63.5+/− 36.6), in Treg cells. It seems that a higher percentage of Treg cells from newly-diagnosed type 1 diabetic patients have on their membrane several known markers of T cell activation (CD122, HLA-DR, HLA-DQ), but not CD220, compared to Tregs from normals. Furthermore, a lower percent of Treg cells from type 1 diabetic patients show surface markers associated with Treg suppressive activity (CD152, TGFβ), and an even lower percent of Tegs from type 1 diabetics show very high expression of these two markers.
Conclusion: These preliminary results from a small sample encompassing a wide spectrum of ages of onset, identifies a number of different markers not examined thus far in Tregs in human diabetes (membrane CD220, HLA-DQ, and TGFβ), or establishes their presence where conflicting results had been obtained previously (very low percentage of Tregs with membrane CD152). Of these markers, HLA-DQ, TGFβ and CD152 have been associated with suppression/regulation of the immune response, and their role in type 1 diabetes pathogenesis requires further investigation.
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0459

The prognostic value of the immunological phenotype of lymphocytes in subjects positive for diabetes-associated autoantibodies

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Background and Aims: The changes of the immunological phenotype of the lymphocytes were documented in the subjects with preclinical stages of type 1 diabetes mellitus. However, the prognostic value of these changes remains unknown. Therefore, the aim of this study was to investigate the immunological phenotype of lymphocytes in subjects positive for diabetes-associated autoantibodies who later developed the clinical type 1 diabetes.
Materials and Methods: We studied 366 normoglycemic children aged 8–15 year old who had first-degree relatives with type 1 diabetes. Fifty nine subjects were positive for autoantibodies against GAD and IA-2 confirmed by two consecutive determinations by radioimmune assays. The control group consisted of 58 age-matched healthy subjects. The children were prospectively followed for 7 years. The immunological phenotype of lymphocytes was analyzed by fluorescent-activated flow cytometry.
Results: During the follow-up type 1 diabetes mellitus manifested in 29 out of 59 subjects positive for diabetes-associated autoantibodies. In this particular group of patients we found the most significant decrease of the number of CD3+lymphocytes ($0.59+0.05.10^9$/L), CD4+ cells ($0.38+0.03\cdot10^9$/L) and ratio of CD4+/CD8+ lymphocytes (1.42+0.06) compared to the entire group of subjects positive for diabetes-associated autoantibodies (0.79 $+0.05.10^9$/L; $0.52+0.03.10^9$/L, and 1.73+0.05 for CD3+, CD4+, cells CD4+/CD8+ratio, respectively, $p<0.05$) and compared to children negative for diabetes-associated autoantibodies ($1.02+0.06\cdot10^9$/L; $0.67+0.04\cdot10^9$/L, and 1.82+0.04 for CD3+, CD4+, cells CD4+/CD8+ratio, re-

spectively, p<0.05) and healthy subjects (1.39+0.09·10^9/L; 0.98+0.04·10^9/L, and 1.98+0.10 for CD3+, CD4+, cells CD4+/CD8+ratio, respectively, p<0.05). The more decreased number of the lymphocytes measured the shorter was the term till the development of the clinical type 1 diabetes. The changes of CD56+between the groups were not statistically significant.

Conclusion: The determination of the immunological phenotype of the lymphocytes could have a prognostic value predicting the development of clinical type 1 diabetes mellitus in the predisposed subjects.

0460

Different islet cell antibody titer at onset of type 1 diabetes: an association with changes in CD4+T cells subsets and clinical course of disease

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Background and Aims: The predictive value of islet cell antibody (ICA) levels at onset of Type 1 diabetes (T1D) in respect to the clinical course of the disease and the changes in T cell subsets has not yet been elucidated. Therefore, the aim of this study was to compare the two groups of recent-onset T1D showing (1) high ICA titer (≥80 Juvenile Diabetes Foundation (JDF) units) (group A, N=33) and (2) low ICA titer (≤40 JDF U) (group B, N=34) at onset of the disease regarding the following parameters: (a) duration of clinical remission (CR) as a non-insulin requiring state during the clinical course of disease and (b) the changes in regulatory T cell subsets, memory (CD45R0+), naive (CD45RA+) and suppressive (CD25+) CD4+T cells, tested both at the onset in insulin requiring state and in the state of CR

Materials and Methods: ICA levels were determined by indirect immunofluorescence on human pancreatic tissue sections and expressed in Juvenile Diabetes Foundation (JDF) units. The percentages of the CD4+T cell subsets were analysed by using two-color immunofluorescence staining and flowcytometry. CR was defined as optimal metabolic control without insulin lasting>30 days.

Results: We found that in group A, the duration of CR was significantly shorter in group A than in group B (A:189±23 vs B: 407±25 days; p<0.05). Simultaneously, when the analysis of CD4+T cells was concerned, although there was no difference between the groups at onset of disease (CD45R0+:37.1+/−2.2 vs 36.8+/−3.3%, CD45RA+: 23.2 +/−2.2 vs 24.9+/−2.0%, CD4+CD25+: 2.37±0.27 vs 2.40±0.31, p=NS), in the state of CR the percentage of CD4+CD45R0+T cells significantly decreased in group B and it became lower compared to group A (A:28.8+/−2.1 vs B:24.0+/−2.0%, p<0.05). Moreover, in the state of CR, both the percentage of CD4+CD45RA+and CD4+CD25+T cells increased in group B and it became significantly higher than to group A (CD45RA+: A:20.5+/−1.7 vs B:27.9+/−1.4, CD25+: A:2.79+/−0.30 vs B:3.82+/−0.44, p<0.05).

Conclusion: Our results have demonstrated that patients with recent-onset Type 1 diabetes and low ICA titer at onset of the disease have shown longer duration of CR accompanied with the increase in regulatory T cell subsets, naive (CD45RA+) and suppressive (CD25+), together with the decrease in memory (CD45R0+) CD4+T cells in comparison to the patients with initially high ICA titer showing shorter CR. The results imply that the ICA levels at onset of recent-onset T1D might represent a useful predictive marker regarding the forthcoming duration of CR and the changes of regulatory T cell subsets.

0461

Relation of serum cytokine levels and chemokine levels with HbA_{1c}, C-peptide and proinsulin in patients with newly diagnosed type 1 diabetes mellitus. Results from the Hvidøre Study Group

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Background and Aims: Diabetes mellitus type 1 results from immune-mediated destruction of β-cells and is accompanied by altered immune responses. The course of disease after manifestation is characterized by various metabolic parameters. Cytokines and chemokines are thought to play an important role in the pathogenesis of type 1 diabetes and for β-cell function. The aim of the current study was to investigate circulating levels of the classical cytokines IFNγ, IL10 and TNFα and the chemokines MIP-1β and RANTES regarding metabolic parameters 1, 6 and 12 months after diagnosis in children and adolescents with newly diagnosed type 1 diabetes.

Materials and Methods: Serum was obtained from 256 newly diagnosed patients with type 1 diabetes (122 males/ 134 females, aged 9.6 years, range 0.2–16.8 years) recruited from 18 paediatric centers of the Hvidoere study group. HbA1c, stimulated C-peptide and proinsulin (Boost test) were determined by high pressure liquid chromatography

(HbA1c), AutoDELFIA -fluoroimmunoassay (C-peptide) and Sandwich ELISA method (proinsulin). Serum cytokines and chemokines were measured by double sandwich ELISA and multiplex technology. Data were analysed by multiple regression analysis including gender, age, IFNγ, IL10, TNFα in one analysis and CCL4/MIP1β, CCL5/RANTES in a separate analysis as co-variates and non parametric Wilcoxon test and Spearman's correlation analysis. All statistical analyses were performed using SAS version 8.6.

Results: One month after diabetes onset, HbA1c associated positively with CCL5/RANTES ($p<0.0001$) a similar trend was observed with IFNγ ($p=0.06$). C-peptide levels related negatively with CCL4/MIP1β ($p=0.037$) but not with cytokines. During follow up, HbA1c was not related to chemokines analysed but did relate to IFNγ($p=0.048$), IL-10 ($p=0.041$) and TNFα ($p=0.037$) at 12 months.

C-peptide associated positively with IFNγ at 6 ($p=0.06$) and 12 months ($p=0.04$) and negatively with CCL4 ($p=0.018$). Proinsulin associated negatively with CCL4/MIP1β but not with cytokines. Prediction models for residual β-cell function (HbA1c, C-peptide, proinsulin) showed no association of cytokines but several associations with CCL4/MIP1β and CCL5/RANTES (reported previously).

Conclusion: The present study shows a relation of IFNγ and IL10 but not of TNFα with metabolic parameters 1, 6 and 12 months after disease onset. Chemokines did relate to metabolic parameters and were predictive of later C-peptide and proinsulin levels. From the cytokines tested, IL10 and IFNγ correlated with other cytokines/chemokines but not with each other, demonstrating a complex regulation of the immune response possibly related to metabolic parameters and residual beta-cell function.

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0462

Association of immune mediators at diagnosis of type 1 diabetes mellitus with later clinical remission

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Background and Aims: We tested the hypothesis that systemic concentrations of cytokines, chemokines or soluble cytokine receptors predict or accompany (partial) clinical remission in type 1 diabetes (T1D).

Materials and Methods: In a prospective, multi-center study 48 patients with newly diagnosed T1D, and 55 age-matched healthy control subjects were investigated. Blood was initially drawn 3–7 days after the diagnosis and then 3–4 months later. Partial clinical remission was defined by centrally measured HbA1c with a maximum of 7.5% or below and daily insulin dose of maximum of 0.38 IU/kg or below. Systemic concentrations of 17 immune mediators were analyzed in serum or plasma. In addition, autoantibodies against insulin (IAA), IA-2 (IA-2A) and GAD65 (GADA) were quantified.

Results: All 17 immune mediators showed remarkable intra-individual stability in their systemic concentrations over time. As a consequence, remission was not accompanied by changes in mediator levels except for a moderate decrease of IL-1ra concentrations ($p=0.02$) and IL-10 concentrations ($p=0.01$) in non-remitters. Low IFNγ($p=0.01$), low IL-10 ($p=0.03$) and low IL-R1 ($p=0.009$) concentrations were associated with later remission. Lower autoantibody titers were also predictive of remission ($p=0.007$).

Conclusion: We conclude that the systemic immunoregulatory state at diagnosis of T1D is predictive of later partial remission. However, partial clinical remission does not appear to be accompanied by a general change in systemic immune reactivity.

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PS 23

Alpha and delta cell function

0463

IL-6 regulates alpha-cell mass and function

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Background and Aims: Increased alpha-cell mass and hyperglucagonemia contribute to hyperglycemia in type 2 diabetes. However, the reasons for this imbalance in alpha-cell mass remain unclear. IL-6 is elevated in the circulation of obese people, and is a strong predictor of the development of type 2 diabetes. The aim of the current study was to investigate the role of IL-6 in the regulation of pancreatic islet-cell mass and function.

Methods: Detection and localization of the IL-6 receptor (IL-6R) and the gp130 was done by gene array and

immunohistochemistry. The effects of IL-6 on cell turnover and function was assessed in human and mouse islets by Ki67, BrdU and TUNEL techniques and by RIA for insulin and glucagon. Morphometric quantification of endocrine cells was performed on pancreatic sections from wildtype and IL-6KO mice.

Results: Initial tissue gene array analysis revealed that the IL-6R is most highly expressed in the pancreatic islet. In human and mouse islets, we observed mRNA expression of both the IL-6R and the signaling component of this complex, the gp130 receptor. Further, IL-6 caused STAT3 phosphorylation in human and mouse islets, while immunocytochemistry localized this activation to the alpha-cell. In support of this, immunohistochemistry performed on pancreatic sections revealed expression of the IL-6R and the gp130 only in alpha-cells. Exposure of cultivated human and mouse islets to IL-6 for 4 days caused a 3- to 6-fold increase in alpha-cell proliferation with no effect on apoptosis. Furthermore we found that IL-6 regulated glucagon secretion in human and mouse islets (IL-6KO islets were used to avoid influence from endogenous IL-6). High doses of IL-6 resulted in a decrease in glucagon mRNA and secretion, whereas lower doses caused an increase in glucagon secretion. In addition, IL-6 impaired beta-cell secretory function as assessed by glucose stimulated insulin secretion (GSIS); an effect which was reversed using an IL-6 receptor antagonist, and partly by exogenous glucagon. Based on these *in vitro* findings we examined the role of IL-6 in regulating alpha-cell mass *in vivo* in WT and IL-6KO mice. Feeding of a high energy diet for 8 weeks induced a 3-fold increase in alpha-cell area in WT mice compared to animals on chow diet. In contrast, alpha-cell area remained unchanged in IL-6KO mice following high energy diet, despite a similar increase in body weight.

Conclusion: Thus, IL-6 presents itself as a regulator of alpha-cell function and proliferation, and may be responsible for the increase in alpha-cell mass seen in patients with type 2 diabetes.

0464

Regulation by glucose of $GABA_A$ receptor expression in mouse α-cells: role in the intra-islet regulation of glucagon secretion

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Background and Aims: The mechanism(s) by which glucose regulates the release of glucagon remain controversial. Whereas paracrine roles for β-cell factors including insulin or Zn^{2+} ions are implicated in the control of secretion from rat islets, we have recently reported that these are unlikely to be involved in the mouse. Moreover, isolated mouse α-cells respond directly to elevated glucose concentrations with increases in ATP production and suppression of Ca^{2+} oscillations, questioning whether paracrine factors may be required at all to regulate glucagon release in this species. Here, we have investigated whether (a) γ-aminobutyric acid (GABA) plays a role in the glucose-induced suppression of glucagon release from mouse islets or clonal αTC1-9 cells, and (b) expression of the individual α, β or γ subunits of the ionotropic $GABA_A$ receptor complex is regulated in α-cells by glucose in the long term.

Materials and Methods: CD1 mouse islets, isolated by collagenase digestion, or clonal αTC1-9 cells, were cultured for 16 h at 10 or 16 mmol/l glucose respectively before assay of glucagon release during static incubations (1 h). αTC1-9 cells were incubated for a further 24 h in the presence of 1, 3, 10 or 16 mmol/l glucose, prior to RNA extraction (Trizol reagent; Sigma). Levels of mRNA encoding $GABA_A$ receptor subunits were determined by quantitative real-time PCR (SYBR green), normalised to 18 S ribosomal RNA. Immunocytochemical analysis was performed after paraformaldehyde fixation and laser-scanning confocal microscopy. Data are expressed as mean-SEM and statistical significance assessed by ANOVA and Newman-Keuls test.

Results: Exogenously added GABA (10 or 100 μmol/l) inhibited glucagon release by 41±11 or 56±5% ($n=4$, $p<0.05$) respectively, during incubation of isolated mouse islets in the presence of 0.5 mmol/l glucose, compared to 63±7% ($p<0.05$) inhibition achieved at 10 mmol/l glucose alone. Treatment with the selective $GABA_A$ receptor antagonist SR95531 was without effect on glucagon release at 0.5 mmol/l glucose but substantially (76.6±20%) reversed the inhibition of glucagon release in response to 10 (*versus* 0.5) mmol/l glucose. Mouse islets expressed α_4, β_3 and γ_2 $GABA_A$ receptor subunit mRNAs, with the immunoreactivity of each largely confined within the islet to the plasma membrane of α-cells. These subunits, plus low levels of α_3, β_2 and δ, were also present in αTC1-9 cells. After culture of αTC1-9 cells for 24 h at 16 *versus* 1 mmol/l glucose, levels of mRNA encoding α_4 were increased 2.7-fold, β_3 by 2.0-fold and γ_2 by 1.6-fold. Correspondingly, culture at 16 (*versus* 1) mmol/l glucose augmented α_4 immunoreactivity in αTC1-9 cells by>two-fold. These effects of high glucose concentration were mimicked at 1 mmol/l glucose by blockade of L-type Ca^{2+} channels with nimodipine. Conversely, stimulation of Ca^{2+} influx with 25 mmol/l KCl reversed the activating effect of 16 mmol/l glucose.

Conclusions: (a) Release of GABA from neighbouring β-cells contributes ~45% of the inhibitory response to glucose of glucagon secretion from mouse islets, whilst direct metabolic effects on α-cells may explain the remaining

effects of the sugar; (b) the expression of α-cell $GABA_A$ receptors displays substantial plasticity. Increased expression of the $GABA_A$ receptor subunits by elevated glucose concentrations may provide a mechanism to suppress glucagon release chronically during sustained hyperglycemia.
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0465

The alpha cell in type 2 diabetes: studies in human pancreatic islets

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Background: In vivo studies have suggested that in Type 2 diabetes mellitus (T2DM) a relative hyperglucagonemia exists and that altered insulin:glucagon molar ratio, contributes to impairment of glucose homeostasis. Little is known, however, of the alpha cell activity within human pancreatic islets. Therefore, we evaluated cell composition as well as insulin and glucagon release from pancreatic islets obtained from normal and type 2 diabetic (T2DM) subjects.
Materials and Methods: Pancreatic islets were prepared by collagenase digestion and density gradient purification from 22 pancreata of multiorgan donors (14 non-diabetic, ND: 8F/6M; age, 68±11 yrs; BMI 23.9±4.1 kg/m2; and 8 T2DM: 6F/2M; age, 66±9 yrs; BMI: 27.0±3.2 kg/m2). Isolated islets were cultured for 24 h with or without 22.2 mmol/l glucose or 1.0 mmol/l FFA mixture (oleic: palmitic, 2:1). T2DM islets were studied within 3 days from isolation. At the end of each incubation, we determined insulin and glucagon release (IRMA or RIA technique), gene expression (qualitative and quantitative RT-PCR) and electron microscopy structure.
Results: With respect to ND, beta-cells were moderately though significantly reduced in T2DM islets (69±3 vs. 62±4%, $p<0.05$) whereas there was no difference in alpha-cell composition (21±3 vs. 23±4%) resulting in relative increase in alpha-cell population. Basal insulin release was reduced in T2DM (220±51 vs. 2531±776 μU/ml/24-h), whereas glucagons was higher (51.5±9.2 vs. 7.3±1.3 pg/ml/24-h; $p<0.001$). In T2DM, glucagon and gene expression was not different with respect to ND. When expression of controlling factors for alpha-cell differentiation was determined, we found no difference in CDX2 while PAX-6 (1.0±0.1 vs. 0.46±0.06) and Foxa-1 (0.43±0.09 vs. 0.24±0.08) were increased. 24 h exposure of ND to FFA, but not to glucose, caused an increase of glucagon release (47.3±12.5 and 6.4±0.9 pg/ml, respectively) that was associated with concomitant increase in PAX-6 (0.7±0.02) and Foxa-1 (0.89±0.2).
Conclusions: These data confirm relative increase in the alpha cell population and enhanced function in T2DM pancreatic islets and suggest, for the first, time an activation of the a-cell lineage both in T2DM pancreatic islets and in ND islets in response to FFA.

0466

Adrenaline but not GLP-1 shifts the voltage-dependence of exocytosis in mouse pancreatic A-cells

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Background and Aim: Glucagon is released from the pancreatic A-cells through Ca^{2+}-dependent exocytosis and it has been demonstrated that GLP-1 and adrenaline stimulate exocytosis in the A-cell through cAMP-dependent mechanisms. However when glucagon secretion is measured from whole islets adrenaline is stimulatory whereas GLP-1 is inhibitory. The aim of this study was to investigate the differences in the mechanisms by which GLP-1 and adrenaline stimulates exocytosis in pancreatic A-cells.
Methods: Single mouse A-cell exocytosis was monitored as increases in cell capacitance using the perforated whole-cell configuration of the patch-clamp technique. Exocytosis was elicited by application of voltage-clamp depolarization of indicated durations from −70 mV to membrane potentials between −30 mV to 20 mV to investigate the voltage-dependence of exocytosis. Glucagon secretion was measured using RIA.
Results: Adrenaline stimulated glucagon secretion in the presence of 1 mM glucose (1G) from 33±1 pg/islet/h (n=10) to 62±3 pg/islet/h ($P<0.001$, n=10), whereas GLP-1 inhibited glucagon secretion to 16±1 pg/ilset/h ($P<0.001$, n=10). The same was observed under depolarizing conditions (in presence of 15 mM K^+) and here adrenaline stimulated glucagon secretion 4-fold ($P<0.001$, n=10) whereas GLP-1 had neither a stimulatory or inhibitory effect. At even higher depolarizing conditions (70 mM K^+) adrenaline stimulated exocytosis 2-fold ($P<0.001$, n=10) and GLP-1 had a slight stimulatory effect (25% increase, $P<0.05$, n=10). To further investigate these differences we investigated the voltage-dependence of exocytosis under control conditions and 4 min after application of either 10 nM GLP-1 or 5 μM adrenaline. Maximal exocytotic response under control conditions was

observed when the membrane potential was depolarized to 0 mV and amounted 39±9 fF (n=5). Addition of adrenaline was associated with a>2-fold (n=5;P<0.01) increase in the exocytotic response. Interestingly, a significance increase in the exocytotic response (13±5 fF, P<0.01 *vs* control) was observed in the presence of adrenaline already at a voltage as negative as −20 mV whereas no capacitance increase was observed at control conditions. In contrast to adrenaline GLP-1 failed to stimulate exocytosis at −20 mV. In the presence of GLP-1 exocytosis was first evoked at the −10 mV depolarization where it amounted 40±11 fF (n=7; P<0.01 *vs* control) Thus, adrenaline stimulates exocytosis at more negative membrane potentials than GLP-1. This might be of importance since small changes in the membrane potential are important in the control of glucagon secretion. The observed shift in the presence of adrenaline but not GLP-1 might be due to that adrenaline stimulate both PKA-independent (through cAMP-GEFII) and PKA-dependent pathways, whereas GLP-1 mainly acts through PKA-dependent stimulation. When testing the effects of the specific cAMP-GEFII-agonist 8CPT-2Me-cAMP using the standard whole-cell configuration it was found to stimulate exocytosis already at −20 mV (8-fold compared to control; P<0.01, n=7–8).

Conclusion: We suggest that 1) adrenaline is able to shift the voltage-dependence of exocytosis though activation of the cAMP-GEFII dependent pathway and 2) the fact that adrenaline stimulates exocytosis at a lower membrane potential than GLP-1 contributes to that adrenaline but not GLP-1 stimulates glucagon secretion from whole islets.

0467

High concentrations of glucose unexpectedly stimulate glucagon secretion by a Ca^{2+} independent mechanism

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Background and Aims: Diabetes is primarily due to insufficient secretion of insulin but is aggravated by a dysregulated release of glucagon. Glucagon secretion is not appropriately stimulated when the blood glucose concentration falls below the fasting level, making diabetic patients treated with insulin or sulphonylureas susceptible to dangerous hypoglycemia. Although glucose is an inhibitor of glucagon secretion patients with diabetic hyperglycemia have elevated levels of glucagon and even respond with additional secretion when blood glucose is raised further. The present study was undertaken to clarify the signal transduction underlying glucose regulation of glucagon secretion.

Materials and Methods: Glucagon and insulin secretion were measured in parallel from C57BL/6 mouse pancreatic islets and glucagon secretion from clonal hamster In-R1-G9 cells using radioimmunoassay. Single mouse islet cells were prepared and used for recording the cytoplasmic Ca2+ concentration with the indicator fura-2 and digital imaging technique. After each experiment the α-cells were identified by immunostaining.

Results: Glucose inhibited glucagon secretion from mouse islets with maximal effect at about 7 mM, which corresponded to the threshold for stimulation of insulin release. Higher concentrations of glucose stimulated insulin release and diminished the inhibition of glucagon secretion. At 25–30 mM glucose there was a paradoxical stimulation of glucagon secretion coinciding with maximal insulin release. Also glucagon secretion from the In-R1-G9 cells showed a U-shaped dependence on glucose concentration with maximal inhibition at 7 and stimulation at 12–20 mM of the sugar. Glucagon secretion stimulated by the absence of glucose was inhibited by diazoxide hyperpolarization but this agent tended to further enhance secretion evoked by high concentrations of the sugar. Studies of isolated mouse α-cells indicated that glucose inhibited glucagon secretion by lowering the cytoplasmic Ca2+concentration. However, stimulation of glucagon release by high glucose concentrations did not require elevation of Ca2+.

Conclusion: Glucose inhibition of glucagon secretion from mouse islets does not require release of factors from the β-cells. High glucose concentrations stimulate glucagon secretion by a novel Ca2+-independent mechanism. An U shaped dose-response relationship for glucose-regulated glucagon secretion may explain why diabetic patients with pronounced hyperglycemia display paradoxical hyperglucagonemia.

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0468

The α-cell expresses GLP-2-receptors and GLP-2 stimulates glucagon secretion

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Background and Aims: In spite of being encoded by the same gene and being secreted by the same type of endocrine cell, glucagon-like peptide-1 and -2 (GLP-1, -2) differ markedly in their effects. Unlike GLP-1, the actions of GLP-2 are regarded as being strictly confined to the gut, where GLP-2 influences intestinal growth, gastric transit and nutrient absorption, while no effect on the endocrine pancreas has been described so far. Recently however,

GLP-2 was reported to stimulate glucagon secretion when given intravenously to healthy human beings, thereby evoking exactly the opposite effect of GLP-1, which potently inhibits glucagon secretion. Since the mechanisms underlying this effect have not been elucidated, our study aimed to explore the mode of action by which GLP-2 increases glucagon secretion. Furthermore, we investigated the effect of GLP-2 on insulin and somatostatin secretion and we examined the impact of simultaneous application of GLP-1 and GLP-2 on glucagon output.

Material and Methods: In order to characterize the GLP-2-mediated effects on islet hormone secretion, the pancreata of male Wistar rats were perfused in situ at a glucose concentration of 3 mM. The glucagon, insulin and somatostatin responses to increasing concentrations of GLP-2 (0.1 to 10 nM, n=8) alone and to combined GLP-1 (0.1 and 1 nM) and GLP-2 (10 nM, n=10) administration were analysed by radioimmunoassay. The localization of GLP-2 receptors (GLP-2r) in tissue samples from human and rat pancreas were studied by immunohistochemistry, employing validated antibodies reliably staining for the GLP-2r and proglucagon, respectively. Total RNA was obtained from isolated rat islets using Trizol and cDNA was created using Moloney Murine Leukemia Virus reverse transcriptase. The expression of the GLP-2r was analysed using primers designed with no crossreactivity with Beacon design Premierbiosoft. The PCR products were analysed on prestained ethidium bromide agarose gel.

Results: In the in situ perfused rat pancreas, infusion of 10 nM GLP-2 caused a significant increase in glucagon secretion (0.5±0.09 vs. 0.27±0.03 pmol/min basal secretion, mean±SEM; $p<0.0001$), whereas lower GLP-2-doses were ineffective (0.3±0.05 for 0.1 nM and 0.36±0.06 pmol/min for 1 nM; p=ns). Neither insulin nor somatostatin output were influenced by the administration of 0.1 to 10 nM GLP-2. The glucagonotropic effect of GLP-2 was not attenuated by the simultaneous administration of 0.1 or 1 nM GLP-1, which when given alone inhibited glucagon secretion by 35.3±7.5 ($p=0.045$) and 42.7±5.7% ($p=0.019$), respectively. Using immunohistochemical double staining, we found that GLP-2r-immunoreactivity was localized to cells which also were immunoreactive for proglucagon in both human and rat pancreas. Expression of GLP-2r transcript was confirmed by PCR on cDNA from isolated rat islets. The result was verified by a PCR-product of the expected length.

Conclusion: The glucagonotropic effect of GLP-2 on the endocrine rat pancreas is mediated by GLP-2r expressed by the α-cell. Based on our analogous findings in human pancreas, this mode of action applies in all likelihood also to human beings. However, further *in vivo* studies are required in order to assess the physiological significance of the glucagonotropic effect of GLP-2 in humans, and to evaluate whether this contributes to the diabetic hyperglucagonemia.

0469

The insulin tolerance test as a method for *in vivo* assessment of α-cell responsiveness in rats

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Background and Aims: Although there is evidence suggesting α-cell dysfunction contributes to the pathogenesis of type 2 diabetes, the regulation of glucagon secretion remains little studied *in vivo* in experimental animals. In humans, the hyperinsulinemic, stepped hypoglycemic clamp is the most rigorous method for quantifying *in vivo* α-cell responsiveness. This method is not readily adapted for rodents. As glucagon release from α-cells serves to protect against hypoglycemia, we have therefore explored the possibility of using the insulin tolerance test as a simple provocative challenge to assess counter-regulatory α-cell responses to hypoglycemic stress.

Materials and Methods: Normal Wistar rats (aged 10–12 weeks) were instrumented with catheters in the jugular vein and carotid artery, which were used for intravenous insulin infusion and arterial blood sampling. Rats were allowed to recover for a week prior to experimentation. Animals were fasted either 14–16 hours overnight or 4–6 hours in the morning, before receiving an intravenous bolus of insulin (Novolin R; 0.1 or 0.05 U/kg). Plasma glucose levels were measured using the glucose oxidase method (YSI) before giving insulin then every 2 minutes for the first 20 minutes and at 25, 30 and 45 minutes after the bolus injection. Blood samples (0.4 ml) were collected at times 0, 4, 6, 8, 10, 12, 16 and 30 minutes for human insulin and glucagon assays (LINCO).

Results: Both doses of intravenous insulin elicited robust decreases in plasma glucose and increased circulating glucagon levels. In animals fasted overnight the baseline glucagon levels were not different (48±3 and 45±2 pg/ml), and 0.1 and 0.05 U/kg insulin elicited similar rates of glucose disposal, measured as the slope of plasma glucose between 2 and 8 minutes (Kg, −6.3±0.1 and −5.5±0.4 mg/dl/min respectively, p=0.09), decreased plasma glucose (−46±3 and −43±2 mg/dl, p=0.52) and increased glucagon levels (438±93 and 374±95 pg/ml, p=0.65). In contrast, when fasted for only 4–6 hours the rate of glucose disposal (−8.2±0.9 and −6.0±0.5 mg/dl/min, respectively; $p<0.05$), fall in plasma glucose (−58±3 vs. −44±2 mg/dl, $p<0.05$) and peak glucagon response (198±27 vs. 95±10, $p<0.005$) were significantly increased with the higher insulin dose. As for total glucagon output, the responses after the overnight fast were similar (135±27 and 117±30 pg/ml*hr, p=0.68) for 0.1 and 0.05 U/kg insulin, whereas after the

short term fast responses were significantly different (60±6 and 32±2 pg/ml*hr, p<0.001).
Conclusion: The hypoglycaemia induced during the intravenous insulin tolerance test elicits an α-cell response leading to a robust increase in circulating glucagon. The α-cell response to, and the ability of glucagon to counteract, the insulin induced hypoglycemia is influenced by the duration of fasting. Overnight fasting led to a larger overall increase in glucagon independent of insulin dose; moreover plasma glucose levels failed to return to baseline and the glucagon levels remained elevated. We speculate the failure of glucagon to alleviate hypoglycemia is due to depletion of liver glycogen stores. Thus, the response following an overnight fast may reflect the maximal α-cell response to hypoglycemia and could be useful to assess insufficient α-cell function. With short term fasting it is possible to demonstrate a dose-dependent glucagon response reflected in both the rate and magnitude of fall in glucose, and the magnitude in the glucagon response, leaving a window for assessing mechanism(s) of enhanced α-cell function *in vivo*.

0470

Glucose stimulates glucagon release in children and adolescents with new onset type 1 diabetes 12 months after diagnosis – results from the Hvidøre Study Group

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Background and Aims: Existance of a glucose-sensing insulin secretion-coupling mechanism similar to beta-cells including the ATP-sensitive K^+- channel Kir6.2 and its regualtory subunit SUR1 was recently demonstrated in alpha-cells isolated from rat pancreatic islets: glucose, arginine, and the sulfonylurea compound tolbutamide all stimulated glucagon release. In humans and intact islets glucagon secretion in response to these agents is suppressed by the simultaneous intra-islet release of insulin. Type 1 diabetes, because of the loss of insulin producing beta cells, offers ideal conditions for an *in vivo* study of glucose-induced glucagon secretion, so the aim of the present study was to investigate the relationship between postprandial glucose and glucagon release in children during the first 12 months after diagnosis of type 1 diabetes. Furthermore we investigated the co-localization of Kir6.2 and SUR1 proteins with glucagon in the human islets.
Materials and Methods: 257 children and adolescents aged <16 years from 22 centres in 18 countries with newly diagnosed type 1 diabetes were followed for 12 months. A 90-minutes Boost-test (mixed meal) was carried out in each patient at 1, 6, and 12 months after diagnosis to characterise the residual beta cell function (C-peptide, and rise in post-prandial blood glucose) and glucagon. All blood substances were centrally determined. Immunohistochemistry was carried out on formalin fixed material. Human pancreatic tissue (n=6) was archival material collected during the 1970s according to the contemporary ethical guidelines. The antibodies used for immunohistochemistry were: monoclonal mouse anti-GLP-1 antibody code GLPb-17F1A37B22C4 (F1) diluted 1:3000, goat anti Kir6.2 code N-18 diluted 1:3000 (Santa Cruz Biotechnology), goat anti SUR1 code C-16 diluted 1:1000 (Santa Cruz Biotechnology)
Results: Multiple regression and compound symmetric repeated measurement models showed that postprandial (90 min post boost-test) glucagon increased over 1, 6, and 12 months (p=0.005) independent of age, gender, stimulated C-peptide (residual beta-cell function), but highly dependent on the rise in postprandial glucose (p=0.0004). Immunohistochemical staining of human pancreatic sections confirmed the co-expression of Kir6.2 and SUR1 proteins in glucagon immunoreactive cells.
Conclusion: The stimulatory effect of glucose on the alpha cells contrast with the suppressive effect of glucose in subjects with preserved insulin secretory capacity and highlights the primary importance of islet paracrine signaling in the regulation of glucagon release. The presence of Kir6.2/SUR1 on alpha-cells suggest that sulfonylureas administered at bedtime to C-peptide negative type diabetic 1 patients might prevent episodes with nocturnal hypoglycemia.
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0471

R-type Ca^{2+} channel-evoked Ca^{2+}-induced Ca^{2+} release regulates somatostatin secretion

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Background and Aims: Somatostatin is a powerful paracrine regulator of insulin and glucagon secretion in the islets but little is known about the control of its release from the δ-cells. Here we have studied the regulation of somatostatin release from mouse pancreatic islets focusing on the role of ATP-regulated K^+-channels, the Ca^{2+}-channel dependence and the importance of intracellular Ca^{2+} stores in the process.
Materials and Methods: Membrane currents and exocytosis were recorded from superficial δ-cells in intact pancreatic islets using the standard whole-cell configuration of the patch clamp technique. δ-cells were identified based on the inactivation of voltage gated Na^+ current and lack of TEA resistant K^+ current. Exocytosis was detected as changes in cell capacitance. For ratiometric Ca^{2+} measurements, indo-1 was infused to the cytosol through the patch pipette. Hormone release from static incubations of groups of ~10 islets and was quantified by radioimmunoassay (RIA).
Results: The L-type Ca^{2+} channel blocker nifedipine (25 μM) inhibited δ-cells Ca^{2+} current by 40% (n=5, $p<0.05$), while it had no effect on somatostatin secretion (n=10). The R-type Ca^{2+} channel blocker SNX482 (100 nM) blocked 45% of the δ-cell Ca^{2+} current (n=4, $p<0.05$) and 80% of glucose-evoked somatostatin secretion (n=10, $p<0.05$). In capacitance measurements, SNX482 blocked 41% of the exocytosis evoked by 500 ms depolarizations from −70 mV to 0 mV (n=4, $p<0.05$). We explored the involvement of calcium-induced calcium-release (CICR) in somatostatin secretion. Exocytosis was evoked by a depolarization of 200 ms from −70 mV to 0 mV, 50 μM Ryanodine, 25 μM dantrolene and 10 μM thapsigargin inhibited exocytosis by 96% (n=4, $p<0.05$), 61% (n=7, $p<0.05$) and 60% (n=4, $p<0.05$), respectively. Similar effects were observed on somatostatin secretion measured by RIA. Blocking CICR by applying ryanodine both intracellularly and extracellularly resulted in a shortening of the $[Ca^{2+}]_i$ transient triggered by 300 ms depolarization, and the time constant for the decay fell from 2.5 s to 1 s (n=5, $p<0.05$).
Conclusion: Based on our data, we conclude that L-type Ca^{2+} channels contribute to the δ-cell Ca^{2+} current without apparent function in δ-cell exocytosis. The major Ca^{2+} sources for exocytosis in δ-cells are influx through voltage gated R-type Ca^{2+} channels and calcium-induced calcium-release via ryanodine receptors in the ER.
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PS 24

Developmental biology of the endocrine pancreas

0472

The long acting GLP-1 analogue, liraglutide, induces beta cell differentiation during normal human pancreas development

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Background and Aims: Glucagon-like peptide-1 (GLP-1) stimulates glucose-dependent insulin secretion; the primary reason long-acting analogues have been developed for use in Type 2 diabetes. In rodents and tumour cell lines, GLP-1 signaling has also increased beta cell 'mass' by promoting beta cell neogenesis, proliferation and inhibiting apoptosis. However, little is known about the role of GLP-1 during normal human beta cell differentiation. We have investigated GLP-1 signaling, via the long-acting analogue Liraglutide (Novo Nordisk A/S) in the developing human pancreas.
Materials and Methods: With ethical approval and informed consent, human fetal material was collected from first trimester termination and processed for fixed tissue, RNA and protein analysis. In vitro culture models were established to interrogate beta cell differentiation. Insulin-positive cells were expressed as a percentage of total epithelial cell number.
Results: Human pancreas was isolated at 8 weeks post-conception (wpc), immediately prior to significant beta cell differentiation (Piper et al, J Endocrinology, 181, 11–23, 2004). GLP-1, along with its receptor, was expressed in the developing pancreas and duodenum. In 7-day explant culture, Liraglutide increased insulin-positive cell number by 68% (n=6), but had no effect on beta cell proliferation or apoptosis. Consistent with this implication of de novo differentiation, exposure to Liraglutide decreased epithelial progenitor cell proliferation by 24%. The effects of Liraglutide on beta cell number were abrogated by co-incubation with 10-fold molar excess of the GLP-1 receptor antagonist, Exendin 9–39, restoring insulin-positive cell number to just below 100% of control. The use of Exendin 9–39 alone reduced insulin-positive cells by 26%.
Conclusion: Liraglutide increases human primary beta cell number, via GLP-1 receptor signaling that most likely enhances beta cell differentiation. For Exendin 9–39 to decrease insulin-positive cell number by itself

suggests that native GLP-1 signaling is very important during early human development. Furthermore, our data suggest that altering the cell cycle status of progenitor cells may be an underlying mechanism that regulates human beta cell differentiation. Clinically, Liraglutide may increase human beta cell mass in Type 2 diabetes. In addition, modulating the GLP-1 pathway may be a potent mechanism for manipulating stem cells to beta cells as an ambitious transplantation therapy in Type 1 diabetes.

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0473

Expression of the Notch-Hes1 pathway in adult pancreatic stem cells

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Background and Aims: In previous studies we have demonstrated that stem cells can be isolated from adult pancreas (APSCs). The aim of the present study was to investigate the mechanisms of stem cell proliferation and differentiation into insulin-producing cells.

Materials and Methods: The expression of stem cell associated markers (BCRP1, nestin, Sox, CK19), markers of progenitor cells (Pdx1, Ngn3, Nkx6.1, Pax4) and proinsulin were analysed in two murine stem cell lines (mI5 and mH1) by flow cytometry and RT-PCR before and after the induction of differentiation. In addition, we studied gene expression programmes known to be involved in the regulation of proendocrine genes.

Results: Both the mI5 and the mH1 cells were positive for nestin, BCRP1, Sox1 and the beta cell progenitor marker Pdx1. By changing the cell culture conditions, it was possible to induce the expression of Ngn3, as well as proinsulin I and II. However, Ngn3 and insulin mRNA and protein expression was low as compared to primary isolated islet cells. Analysis of the Notch signaling pathway revealed that Notch1 and Hes1 were highly expressed under basal condition and were only slightly decreased after the differentiation phase.

Conclusion: We here demonstrate for the first time that the Notch-Hes1 pathway is activated in APSCs. It is known that Hes1 strongly suppress Ngn3 expression. This could be one explanation for the incomplete *in vitro* differentiation and the low number of insulin positive cells. Thus, inhibition of Hes1 expression could be a target to further improve the *in vitro* generation of insulin-producing cells from adult stem cells.

0474

Human exocrine pancreas-derived mesenchymal stem cells and their potential to differentiate into beta cells

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Background: Transplantation of *in vitro* generated islets or insulin producing cells represents an attractive option for treatment of type 1 diabetes. Therefore, stem or progenitor cells with the capacity to differentiate into beta cells have to be identified. In this study we isolated and expanded mesenchymal stem cells (MSC) obtained from human exocrine pancreas and we investigated their potential to differentiate into beta cell.

Methods: We have cultured human exocrine pancreatic tissue obtained after isolation and purification of pancreatic islets for clinical transplantation in expansion media for human MSC (IMDM+10% FCS+PDGF-BB). After 2–8 passages, these cells were characterized by FACS and compared to human MSC isolated from bone marrow. Mesenchymal differentiation potential was tested by culturing these cells in adipogenic and chondrogenic differentiation media. In order to induce endocrine differentiation, these cells were cultured at high density on non-adherent plastic in differentiation medium (high glucose DMEM containing nicotinamide, activin A and HGF). Differentiation was assessed by RT-PCR for insulin and early endocrine markers.

Results: In 14 of 18 human pancreatic exocrine fractions with a purity of 99%, adherent fibroblast-like cells appeared and could be expanded. Cells were grown up to 40 population doublings (19 passages). These cells displayed a similar antigen surface expression as bone marrow MSC, i.e. they were negative for CD31, CD34, CD45, CD106, MHC class 1, CD54low, and positive for CD44, CD90, CD105. Culture of these cells in adipogenic and chondrogenic differentiation media allowed differentiation into adipocyte-like and chondrocyte-like cells, demonstrating mesenchymal phenotype and multipotentiality. Pancreatic MSC, when cultured in differentiation medium formed pseudo-islet-like clusters and after 15 days of differentiation expressed Pdx1, Nkx2.2, Nkx6.1, NeuroD, Isl-1, insulin, glucagon and glucokinase in 6 out of 10 experiments. However, these islet-like clusters were negative for Glucokinase and Glut2.

Conclusion: Our data show that MSC are present in the exocrine fraction of human pancreas and that they can be

expanded extensively. These cells have the potential to form islets-like clusters expressing several early and late beta-and alpha-cell genes.
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0475

Endothelial cell involvement in proliferation and differentiation of human pancreatic duct cells

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Background and Aims: Signals from vascular cells are required for normal pancreatic development. We hypothesized that endothelial cells modulate human duct cell proliferation and differentiation into a beta-cell phenotype.
Materials and Methods: To test this hypothesis we isolated human duct cells from tissue remaining after islet isolation. The islet-depleted tissue was dispersed by EDTA/trypsin followed by immunomagnetic cell sorting using antibodies against the duct marker CA19-9. CA19-9+cells were expanded as monolayers during culture in DMEM medium plus 10% fetal bovine serum (expansion phase). After 5–7 days cells were dissociated and transferred into hydrophobic multiwell plates and aggregated in serum-free DMEM medium with ITS and nicotinamide for 5–6 days (differentiation phase). Bovine aortic endothelial cells (BAEC) were plated onto Transwell inserts and used at 50–70% confluency for co-culture experiments during the expansion and differentiation phases. Every other day new Transwell inserts were used. For qRT-PCR 10 ng RNA equivalent was used.
Results: Ninety percent of dispersed cells were positive for CK19 and no insulin+cells were detected. Endothelial cells facilitated rapid attachment of dispersed CA19-9+cells and increased proliferation (25.6±6.9 vs 17.0±2.9% Ki67+cells after 2 days co-culture, mean±SD, $p<0.05$; n=5). All Ki67+ cells were positive for CK19 by double immunofluorescence assessment. Cycle threshold (C_T) values for insulin and IPF1 gene expression were 30.3±3.7 and 28.3±0.7, respectively (n=5–8) in sorted CA19-9+cells by qRT-PCR. Insulin gene expression decreased 5.6 fold during expansion and increased 35.4 fold during aggregation compared to sorted CA19-9+cells. No significant change was observed in IPF1 gene expression during the expansion and differentiation phases (C_T 28.2±1.5 and 28.6±0.6, respectively). Endothelial cell co-culture attenuated the increased insulin gene expression during the differentiation phase (only 4.1 fold increase, $p<0.01$ vs control) and resulted in a non-significant decrease in IPF1 expression (C_T 30.1±1.5; n=3). Endothelial-cell conditioned medium gave similar results.
Conclusion: Soluble factors from endothelial cells stimulate proliferation but attenuate differentiation of CA19-9+ human pancreatic duct cells.
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0476

Identification of adult stem/progenitor cells in human exocrine pancreas

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Background and Aims: In recent years, evidence is provided that tissue-specific stem cells reside in certain adult organs; yet their specific properties remain elusive because of their rarity, technical difficulty in identifying them and characterizing their progeny. Nevertheless, while haematopoietic stem cells can be isolated nowadays using panels od surface markers, such strategies have not been convincingly applied to stem cells of most compact organs. With regard to the endocrine system, several potential adult islet/beta cell progenitors derived from pancreatic islets, liver and bone marrow have been studied, but, to date, no candidate cell has been fully characterized or is clinically applicable. Beside haematopoietic stem cells, neural stem cells, seem to be the cells most convincingly characterised. In this study, we aimed to device a method for isolating stem/progenitor cells from the human exocrine pancreas.
Materials and Methods: In initial experiments, tissue specimens from human exocrine pancreas were enzymatically digested; selected fragments, residual to digestion were plated and cultured in the presence of EGF and bFGF.
Results: Spheroids with self replicative potential were obtained. Primary spheroids were harvested after 7–10 days; they were mechanically dissociated into single cells and replated for generating secondary cultures. The different

lines of spheroids, generated from different pancreatic specimens, were characterised in flow cytometry (FACS). In addition to elements negative for pancreatic differentiation markers, a population of cells within spheroids was positive for insulin, glucagon, somatostatin, cytokeratin and pancreatic homeobox protein IDX-1. Stem cell markers identified a small percentage of CD34+CD45- cells. The percentage of positive cells varied within the different lines. Spheroids were also studied in RT-PCR; mRNA of nestin, a known marker of neurospheres, was detected. To verify whether spheroids could be induced to produce insulin, replicate cultures from the same spheroid lines were functionally evaluated in 3D collagen gel cultures. While in basal conditions (EGF, bFGF) a low level of insulin production was detectable in culture supernatants, progressively increasing levels of insulin were obtained in the presence of differentiating factors. We also attempted to verify whether spheroids obtained from the exocrine pancreas could acquire phenotypic characteristics of neuronal lineage. We therefore co-cultured CFDA labelled spheroids with the c-kit positive neuroblastoma cell line LAN-5. In FACS analysis, a population of cells expressing c-kit, was obtained. These cells were of a larger size compared with cells in the spheroidal population when kept in basal conditions, but smaller than neuroblastoma cells.
Conclusion: We conclude that a population of stem/progenitor cells exists within the human exocrine pancreas. Our data support the concept of an intrinsic plasticity of the spheroids that showed in functional studies ability to generate insulin-producing cells and potentially non-pancreatic cells.
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0477

Defective IGF-2 and IGF-1R protein expressions in pancreatic rudiment precede beta cell mass anomaly in GK rat

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Background and Aims: The Goto-Kakizaki (GK) rat is a spontaneous model of type 2 diabetes with a defective beta-cell mass detectable in late fetal age. To determine the involvement of IGF-2 in this defect, we evaluated during GK pancreatic organogenesis: 1) the GK beta-cell development on embryonic day (E) 13.5 and E18.5; 2) IGF-2 and IGF-1 receptor (IGF-1R) pancreatic protein expressions at E13.5 and E18.5; 3) the *in vitro* development of E13.5 GK pancreatic rudiment; 4) the effect of IGF-2 addition on beta-cell mass in pancreatic rudiment *in vitro*.
Materials and Methods: GK rats were obtained from our local colony. Wistar (W) rats were used as control. Beta-cell quantitative analyses were determined by immunohistochemistry and morphometry at E13.5, E18.5 and in pancreatic rudiments after culture. IGF-2 and IGF-1R pancreatic protein expressions were evaluated using Western blot analyses at E13.5 and E18.5. For culture experiments, dorsal pancreatic rudiments were dissected at E13.5, separated from its surrounding mesenchyme and cultured for 7 days onto three-dimensional collagen gel at 5.5 mM of glucose. Recombinant IGF-2 (100 ng/ml) was added every day to the culture medium. To label cells in the S phase, BrdU was added to the culture medium one hour before the end of the culture. Apoptosis in pancreatic rudiment was evaluated using the ApopTag kit.
Results: 1) The number of beta-cell was normal in GK pancreatic rudiment at E13.5. However, pancreatic beta-cell mass was decreased by 74% in E18.5 GK as compared to W control at the same age. 2) IGF-2 and IGF-1R protein expressions were respectively decreased by 27% and 42% in E13.5 GK pancreatic rudiments as compared to W controls. Again, IGF-2 and IGF-1R pancreatic protein expressions were decreased (by 58% and 34% respectively) in GK at E18.5 as compared to W values. 3) After 7 days of culture, GK pancreatic rudiments exhibited a beta-cell number reduced by 78%. This beta-cell defect was similar to that observed in E18.5 GK. After culture, the total pancreatic-cell and the beta-cell proliferation rates in GK pancreatic rudiment were decreased respectively by 27% and by 40% as compared to W values. Moreover, the apoptotic rate of total cells was increased by 42% in GK pancreatic rudiment after culture. 4) The daily addition of IGF-2 to GK pancreatic rudiment increased the beta-cell number by 123%, the total cell proliferation rate by 55%, and the beta-cell proliferation rate by 111% as compared to GK pancreatic rudiment cultured without IGF-2.
Conclusion: Taken together these data show that while beta-cell mass is already decreased at E18.5, the differentiation of the first beta cells is in fact normal in E13.5 GK pancreas. Thus, a defective IGF-2 and IGF-1R protein expressions within the GK pancreatic rudiment represent a primary anomaly. The isolated GK pancreatic rudiment as maintained *in vitro* under the conditions here described, mimicks the GK beta-cell deficiency as observed *in vivo*. This *in vitro* approach was useful to highlight the effect of an IGF-2 supplementation on GK beta-cell growth. Moreover, it also suggests that, IGF signaling pathway seems to be functional in GK beta cells in response to IGF-2.
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0478

Diabetes impairs ischaemia and cytokine-mediated mobilisation of progenitor cells in rats

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Background and Aims: Endothelial progenitor cells (EPCs) represent a fraction of bone marrow-derived pluripotent stem cells which can be mobilized to the peripheral circulation in response to many stimuli, including tissue ischemia and cytokines. Once in the peripheral blood, EPCs are involved in cardiovascular homeostasis as they have the potential to repair the damaged endothelium and take part in compensatory neovascularization. Conversely, a decrease in the EPC pool is associated with established atherosclerosis and increased cardiovascular damage. Mechanisms involved in the pathogenesis of diabetic macrovascular complications include impaired angiogenic response to ischemia. Therefore, reduction of circulating EPC level may be a plausible cause of increased cardiovascular risk in diabetes mellitus. Causes of EPC decrease may include shortened peripheral survival and defective bone marrow stimulation. This study was undertaken to test the hypothesis that a weak ischemia- and cytokine-induced bone marrow mobilization is responsible for decrease of circulating EPCs in diabetes. Moreover, possible favorable effects of glucose lowering on EPC mobilization were also assessed.
Materials and Methods: We employed a model of hind limb ischemia-reperfusion (IR) injury in control and diabetic rats, 4 weeks after streptozotocin induction. Peripheral blood EPCs were defined as Sca-1$^+$c-kit$^+$ cells and were counted by flow cytometry. Capillary density was quantified by vWF immunofluorescent staining of muscle sections. Plasma levels of stromal cell derived factor (SDF-1) and vascular endothelial growth factor (VEGF) were measured by ELISA. Protein levels of hypoxia-induced factor (HIF-1α) were determined with Western Blot analysis.
Results: In control animals, EPCs increased after ischemia and described a mobilization curve within 7 days, while diabetic rats were completely unable to mobilize EPCs after IR injury. As a consequence, diabetic rats had no compensatory increase in muscle capillary density, while a 50% increase was found in control rats. Defective EPC mobilization in diabetic animals was associated with altered release of SDF-1 and VEGF and with inability to upregulate HIF-1α in muscles subjected to the IR injury. Insulin administration to achieve near-normal glucose levels during ischemia and reperfusion periods increased VEGF and determined a partial recovery of EPC mobilization in diabetic rats. Administration of a combination of 50 μg/kg granulocyte-colony stimulating factor (G-CSF) and 200 μg/kg stem cell factor (SCF) daily for 5 days stimulated mobilization of EPC in diabetic rats, but to a lesser extent when compared to control rats. However, a premedication based on G-CSF plus SCF was able to promote EPC mobilization after IR injury in diabetic animals.
Conclusion: Defective ischemia- and cytokine-mediated bone marrow mobilization causes reduction of circulating EPCs in diabetes and impairs compensatory angiogenesis in ischemic tissues. Inability to up regulate hypoxia-sensing systems in the target tissue together with an intrinsic bone marrow deficit seems to be responsible for this pathophysiological alteration. Growth factor administration together with blood glucose control may represent in perspective a rational therapeutic strategy for diabetic ischemic syndromes.
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0479

Generalised immaturity of metabolic pathways in neonatal β cells

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Mature β-cells are characterized by glucose stimulated insulin secretion (GSIS) that results from specialised metabolic pathways. Fetal and neonatal β-cells show an impaired insulin secretion in response to glucose but not to aminoacids. Only by 1 week after birth is glucose responsiveness established. The basis of this difference between neonatal and adult β-cells remains unclear. We hypothesize that this unresponsiveness is due to a generalised immaturity of the metabolic pathways found normally in β-cells. Our aim was to compare neonatal and adult β-cells by performing gene expression studies on laser capture microdissected β-cell enriched cores of neonatal (1-day-old) and adult (6-week-old) pancreas (n=4 each). RNA was extracted from the samples and amplified twice using T7 polymerase and hybridized to U34A Rat Genome microarrays. dChip software (2 fold differences, $p<0.05$) was used to analyze the differences between the neonatal and adult samples; 215 genes/ESTs were significantly higher and 375 significantly lower in neonatal than adult β-cell-enriched cores. Among the genes poorly expressed in the neonatal β-cells were key metabolic genes including membrane mitochondrial shuttles (malate dehydrogenase, glycerolphosphate dehydrogenase, aspartate aminotransferase, malic enzyme), pyruvate carboxylase and carnitine palmitoyl

transferase (CPT) II. Interestingly, CPT I was found to be higher in the neonates. These enzymes play key roles in the insulin secretion mechanism. These microarray findings were confirmed by real time PCR on 5 independent samples of isolated neonatal and adult islets (using the $\Delta\Delta C_T$ analysis, 3-13 fold lower in neonatal islets, $p<0.01$). Thus, the lack of glucose responsiveness in neonatal islets may be due to a generalized immaturity of the metabolic specialization of pancreatic β-cells.

PS 25

Transcriptional regulation in beta cells

0480

Mice lacking Hnf1α (MODY3) reveal that gene activity is linked to nuclear compartmentalisation of histone modifications

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Background and Aims: Mutations in the transcriptional activator gene HNF1α (also known as TCF1 or MODY3) are the major cause of monogenic diabetes, and represent a paradigm for a human transcriptional disease. HNF1α is known to induce the acetylation of histones at target chromatin. Histone tail modifications such as acetylation and methylation are thought to be critically important in gene transcription. Recent evidence also indicates that gene regulation is associated with repositioning of loci within the nucleus. However, the possible relationship between histone modifications and nuclear topology concerning gene transcription is uncertain.
Material and Methods: Chromatin immunoprecipitation (ChIP) and confocal immunofluorescence-FISH analysis have been carried out in freshly isolated Hnf1α-deficient and wild-type hepatocytes and islets.
Results: We show that the nucleosomes of genes that become silenced in Hnf1α-deficient cells exhibit reduced methylated H3-Lys4 and increased methylated H3-Lys27. We also show that RNA Polymerase II and trimethylated H3-Lys27 are enriched in mutually exclusive nuclear domains. Remarkably, genes that become silenced in Hnf1α-deficient cells reposition from nuclear domains enriched in methylated H3-Lys4 and RNA polymerase II to domains enriched in methylated H3-Lys27.
Conclusion: This study provides a novel insight into the defect underlying MODY3, and suggests that transcription is linked to histone code modifications not only locally, but also to its representation in nuclear space.
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0481

Effect of aging on the expression of beta cell function-related genes in islets

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Background and Aims: Age-related change in insulin secretory function may contribute to the alterations in glucose metabolism with age. Recently, the glucose-stimulated insulin release of isolated human islets has been shown to deteriorate progressively with advancing donor age. This decline in beta cell function with aging may contribute to the increasing development of IGT and type 2 diabetes and also to the progressive nature of the disease. The aim of this study was to see whether there is any change in mRNA expression of beta cell-function-related genes in islets with aging.
Materials and Methods: Pancreatic islets were isolated from young (2 mo-old) and old (22–24 mo-old) LETO rats and C57BL/6N mice. After overnight culture, *in vitro* glucose-stimulated insulin release was assessed by incubating in medium with either 1.7 or 16.7 mM glucose for 1 hour. Analysis of mRNA levels of beta cell function-related genes was done with real-time RT-PCR with RNAs (n=3–6/group) isolated from pooled islets (3–5 animals/sample).
Results: The *in vitro* glucose-stimulated insulin release index was significantly lower in islets from old mice compared with young mice (2.7 vs. 1.3). PDX-1, insulin, GLUT2 and prohormone convertase 1/3 gene expression in islets was markedly lower in old rats (33%, 13%, 20% and 34%) and old mice (42%, 35%, 19% and 15%) compared with young animals: mRNA expression of Foxa2 and HNF1alpha in islets was similar regardless of mice age. On the other hand, genes not related to beta cell-specific function, such as caspase 3, ATP synthase and glycerol kinase were not significantly different in expression in islets according to age. UCP-2, known to suppress glucose-stimulated insulin release when overexpressed in islets, was not increased in mRNA expression in old animal islets.
Conclusion: With increasing age, insulin secretory function of islets deteriorates accompanied with a decrease in expression of insulin synthesis/secretion-related genes, such as PDX-1, GLUT2 and prohormone convertase.

0482

Cytokines and dsRNA induce expression and activation of HIF-1α in rat pancreatic beta cells

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Background and Aims: Type 1 diabetes mellitus (T1D) is an autoimmune disease characterized by a progressive destruction of pancreatic beta cells. Viral infections and cytokines contribute to the pathogenesis of T1D. Exposure of primary rat beta cells to the viral product double stranded RNA (dsRNA; tested in the synthetic form PIC), in combination with interferon-γ (IFN-γ), induces beta cell apoptosis. By microarray analysis, PIC and/or cytokines were shown to induce expression of several genes and transcription factors, including the hypoxia-inducible factor 1α (HIF-1α) and several genes downstream of this transcription factor, such as Glut-1, VEGF and hexokinase II. HIF-1α is induced during hypoxia and its activated form plays a key role in the regulation of genes implicated in metabolism, cellular proliferation and viability. We presently examined the regulation of HIF-1α in primary rat beta cells and insulin-producing INS-1E cells. PC12 cells were used as positive controls.
Material and Methods: FACS-purified primary rat beta cells, INS-1E cells and PC12 cells were exposed for 30 min, 2, 6, 12 and 24 hours to IL-1β (10–50 U/ml) and/or IFN-γ (100–1000 U/ml) in the presence or absence of PIC (100 μg/ml) and/or the iNOS blocker NMA (1 mM). mRNA expression was examined by real-time RT-PCR and corrected for GAPDH expression. Activation of the HIF-1 promoter was studied by luciferase reporter assay. As positive control for HIF induction, we exposed the cells to cobalt chloride (CoCl2; 150 μM).
Results: Exposure of primary rat beta cells or INS-1E cells to IL-1β induced a 2–3 fold increase in HIF-1α mRNA expression after 24 hours ($P<0.05$; n=8), while treatment with IL-1β+IFN-γ induced an earlier and more pronounced increase in HIF-1α mRNA expression, which was already significant after a 6-hour treatment ($P<0.05$; n=4), and reached the highest levels after 24 hours (16.5 in cytokine-treated vs 5.2 in control beta cells; $P<0.001$; n=5). In primary beta cells, PIC in combination with IFN-γ also induced a significant increase in HIF-1α mRNA expression after 24 hours (20.0 in cytokine-treated vs 6.0 in control cells; $P<0.01$; n=5). The addition of NMA did not affect HIF-1α induction, indicating that HIF-1α expression does not depend on nitric oxide formation. In primary beta cells, exposure to IL-1β+IFN-γ or to CoCl2 activated an HIF-1α luciferase promoter-reporter after a 12-hour treatment (2.5 and 3.6 fold increase respectively ; $P<0.05$ vs control; n=5). In PC12 cells, a 12-hour exposure to CoCl2 also increased activity of the reporter construct (4.8 fold increase ; $P<0.01$ vs control; n=3).
Conclusion: These data indicate that exposure of primary rat beta cells to either IL-1β, IL-1β+IFN-γ or PIC+IFN-γ induces HIF-1α mRNA expression. Exposure of INS-1E cells to IL-1β or to a combination of IL-1β+IFN-γ also induces HIF-1α mRNA. In primary rat beta cells, IL-1β+IFN-γ activates an HIF-1α promoter-reporter, suggesting that HIF-1α is functional in these cells. Induction of HIF-1α expression is probably part of the adaptive stress responses of primary rat beta cells exposed to agents that impair cell metabolism.
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0483

Posttranslational modifications of the transcription factor Sp1 regulate glucose-induced NFκB p65 expression

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Background and Aims: The transcription factor NF-κB plays an important role in diabetes. The molecular mechanisms underlying the observed long-term activation of NF-κBp65 mRNA transcription in diabetes are yet unknown. Detailed characteristics of the p65 promoter shows several binding sites for the transcription factors Sp1/Sp3, implying that these proteins might act as transactivators of p65 *de novo* synthesis under hyperglycemic conditions. While Sp1 expression is not modulated by high glucose, we studied the role of glucose-mediated Sp1 posttranslational modifications. We characterized the influence of Sp1 O-glycosylation and phosphorylation for NF-κB p65 gene transcription regulation under high glucose.
Materials and Methods: Binding capacity of Sp1 and Sp3 was measured in THP-1 and BAEC cells using electrophoretic mobility shift assay (EMSA). Full-length p65 promoter and its deletion mutants lacking Sp1 binding sites were cloned into luciferase reporter vector pGL3 and transfected into THP-1 and BAEC cells. Posttranslation modifications of Sp1 (O-glycosylation, phosphorylation and nitrotyrosine residues) were characterized by immunoprecipitation followed by immunoblotting. The transcription of NF-κB p65, O-GlcNAcase and O-GlcNAc transferase was determined by RT-PCR.
Results: High glucose (30 mM) induced a time- and dose-dependent activation of Sp1/Sp3 binding capacity to consensus Sp1 sequence. Transient transfection of p65 promoter constructs indicated that deletion of every Sp1 binding site significantly impaired NF-κB p65 expression.

Consistently, Sp1-antisense blocked NF-κB p65 mRNA synthesis. Sp1 is constitutively phosphorylated and O-glycosylated, however, Sp1 O-glycosylation was dramatically increased immeadiately after high glucose induction, followed by continuous deglycosylation and increasing phosphorylation. Furthermore, the expression of O-GlcNAc transferase and O-GlcNAcase, enzymes, responsible for Sp1 O-glycosylation, was modulated by high glucose. Maximal expression of O-GlcNAcase, minimal Sp1 O-glycosylation and p65 mRNA *de novo* synthesis were achieved in the same time.

Conclusion: High glucose modulates posttranslational modifications and transactivation capacity of Sp1, which results in NF-κB p65 mRNA *de novo* synthesis in both, monocytic and endothelial cells.

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0484

Calcium entry is involved in 804G-ECM-induced NF-κB activity and actin cytoskeleton remodelling

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Background and Aims: Pancreatic beta cells cultured on a laminin-5 rich ECM (804G-ECM) display increased spreading and improved function (glucose-stimulated insulin secretion). It was shown previously that Ca^{2+} entry is involved in 804G-ECM induced cell spreading, that 804G-ECM induces NF-κB transcriptional activity and that this activity is involved in 804G-ECM-induced beta cell spreading, cytoskeleton remodelling and beta cell function. The aim of this work was to study the involvement of Ca^{2+} entry in 804G-ECM-induced NF-κB activity and in the impact of 804G-ECM on actin cytoskeleton remodelling.

Materials and Methods: Diazoxide (which impairs depolarization and Ca^{2+} entry) and SR-7037 (L-type Ca^{2+} channel blocker) were used to inhibit calcium entry. NF-κB nuclear translocation was assessed by immunofluorescence (after 1 h of culture), and expressed as the number of cells with dominant NF-κB nuclear staining, normalized to control (804G-ECM=100%). NF-κB transcriptional activity was analyzed by quantification of mRNA levels of IκBα, an established NF-κB target gene, relative to L3 RNA levels (normalized to pLL=1) by real-time RT-PCR (4 h of culture). Actin cytoskeleton remodelling was analyzed by confocal immunofluorescence using phalloidin or antibodies specific for β- or γ-actin isoforms. All experiments were performed with primary rat pancreatic beta cells purified by FACS. Data are means±SEM (n=3 independent experiments).

Results: Treatment of beta cells with Ca^{2+} entry blockers reduced 804G-ECM induced NF-κB nuclear translocation significantly (804G-ECM control=100±37.31, 804G-ECM+diazoxide=51.84±11.93 ($p<0.02$ vs control); 804G-ECM+SR-7037=61.43±10.63 ($p<0.03$ vs control)). Furthermore, 804G-ECM-induced overexpression of the NF-κB target gene IκBα was significantly inhibited in cells treated with the Ca^{2+} entry blockers (804G-ECM control=3.3±0.49, 804G-ECM+diazoxide=1.5±0.16 ($p<0.01$ vs control), 804G-ECM+SR-7037=1.6±0.45 ($p<0.03$ vs control)). Actin cytoskeleton remodelling was induced in cells cultured on 804G-ECM and use of actin isoform-specific antibodies revealed that γ-actin is the major remodelled isoform. Furthermore, Ca^{2+} entry blockers diazoxide and SR-7037 both perturb 804G-ECM-induced γ-actin and total actin reorganization.

Conclusion: These results show that Ca^{2+} entry is involved in 804G-ECM-induced NF-κB activity and in 804G-ECM-induced γ-actin cytoskeleton remodelling. Future work is aimed at identifying the link between Ca^{+2} entry and NF-κB activity, that is involved in 804G-ECM-induced actin cytoskeleton remodelling and improved insulin secretion.

0485

Insulin-stimulated c-fos gene transcription in pancreatic β cells requires clathrin-dependent endocytosis of IR-B and signaling from early endosomes

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Background and Aims: Research from over the last seven years provides convincing evidence that beside liver, muscle and fat, also the insulin-producing β-cell is a target for positive insulin action. Here insulin has been shown to be involved in maintaining normal β-cell function by regulating processes such as transcription, translation, ion fluxes, cytoprotection and/or proliferation and, therefore, might be important for maintaining β-cell mass. By studying the insulin-dependent activation of three candidate genes, i.e. insulin, glucokinase (BGK) and c-fos genes, we have previously shown that the two insulin receptor (IR) isoforms IR-A and IR-B contribute to the selective action of insulin. Insulin activates its own gene by signaling through IR-A/PI3K Ia/p70s6k, it activates BGK via IR-B/PI3K-C2α/PKB and c-fos via IR-B/Shc/MEK/ERK. While activation of the insulin and BGK genes occurs from receptors situated in different microdomains of the plasma

membrane, c-fos gene transcription is sensitive to the action of dominant-negative dynamin-K44A suggesting the involvement of receptor endocytosis. The aim of this study was to identify the mechanisms that contribute to dynamin-dependent activation of c-fos gene transcription by insulin.
Materials and Methods: We employed c-fos mRNA analysis, c-fos-promoter-driven reporter gene (GFP or DsRed) expression in online monitoring studies with transient co-expression of effector proteins. To discriminate between caveosome- and clathrin-dependent endocytosis, we studied the effect of co-expressed caveolin forms and that of the negative-interfering form of the AP2 subunit μ2, i.e. μ2-T165A. The involvement of the early and late endosome pools in IR-B-mediated signaling was analyzed by subcellular fractionation and the effect of co-expressed Rab5 and Rab7 variants, small GTP-binding proteins involved in the regulation of membrane trafficking in the endocytic pathway.
Results: Our data show that c-fos gene activation by insulin requires clathrin- but not caveosome-dependent endocytosis of IR-B with the integrity of the juxtamembrane GPLY-motif for receptor internalization. Preparation of early endosomes showed the presence of tyrosine-phosphorylated, i.e. activated, IR-B. The involvement of the early endosome pool but not that of late endosomes in IR-B-mediated activation of c-fos gene transcription was shown by the effect of dominant-negative Rab5a-S34N and Rab7-T22N, respectively.
Conclusion: Our data provide evidence that IR-B mediated activation of c-fos gene transcription in insulin-producing cells requires clathrin-dependent endocytosis of the receptor and signaling from the early endosome pool. These data lend further support to the concept that spatial segregation of selective signaling pathways originating from IR-A and IR-B allows the simultaneous activation of discrete signaling cascades that lead to specific insulin effects.
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0486

Inhibition of the glucose-6-phosphatase gene expression by TNFα requires the activation of IKKβ

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Background and Aims: Type II Diabetics exhibit fasting hyperglycaemia which is in part caused by permanent overexpression of the key gluconeogenic enzymes glucose-6-phosphatase (G6Pase) and PEPCK due to insulin resistance of the liver. Proinflammatory cytokines like TNFα cause a strong inhibition of basal and induced G6Pase transcription and may contribute to the hypoglycaemia observed during the final stages of a sepsis. Recently, we have shown that TNFα represses the G6Pase gene expression via NFκB activation.
Here we analysed whether the IKK complex is necessary for the inhibition of G6Pase gene expression, but on the other hand leads to an insulin resistance in H4IIE rat hepatocytes.
Materials and Methods: Wild type IKKβ (IKKβ-WT), a dominant negative (IKKβ-S177/181A; IKKβ-DN) and a constitutively active IKKβ (IKKβ-S177/181D; IKKβ-CA) were characterised by an *in vitro* kinase assay with IκBα as a substrate. The G6Pase promoter construct (−1227/+57) and a minimal thymidine kinase promoter containing two consensus NFκB binding elements (TK-NFκB) were analysed in H4IIE rat hepatoma cells by promoter reporter gene assays. Endogenous G6Pase expression was assessed by Northern Blot. IKKβ-DN which blocks the activation of the endogenous IKK complex was overexpressed using recombinant adenovirus. Phosphorylation of Proteinkinase B (Akt) in response to insulin with and without TNFα preincubation was detected by a specific phospho Ser473-Antibody in western blots.
Results: To show that IKKβ activity is sufficient to suppress G6Pase gene expression we cotransfected the G6Pase promoter construct together with the wild type IKKβ, IKKβ-DN and IKKβ-CA. In comparison to IKKβ-DN the IKKβ-WT showed a significant inhibition of the G6Pase promoter by 20%. The IKKβ-CA construct was more effective in the suppression of the promoter (50% inhibition) which clearly shows that the activation of IKK is sufficient to suppress basal and induced G6Pase promoter activity. Catalytic activity of each constructs was confirmed by *in vitro* kinase assays. In these assays IKKβ-CA showed the highest activity, IKK-WT showed modest activity whereas IKKβ-DN was completely inactive. The specificity of the effect on G6Pase promoter was shown by using coexpression of the respective constructs with the TK-NFκB promoter construct. As expected, promoter activity corresponds to the activity of the respective IKKβ construct.
Adenovirus mediated overexpression of IKKβ-DN showed a dose dependent inhibition of IκBα degradation after TNFα stimulation. Downregulation of endogenous G6Pase mRNA after TNFα stimulation is significantly inhibited after adenoviral IKKβ-DN overexpression. To further prove that IKKβ is necessary for inhibition of G6Pase gene transcription by TNFα experiments with a specific IKKβ inhibitor are ongoing. Additionally we could not detect a reduced phosphorylation of Akt in response to insulin after TNFα stimulation in H4IIE cells.
Conclusion: These results demonstrate that IKKβ is not only sufficient but also necessary for the suppression of endogenous G6Pase gene transcription and promoter

activity by TNFα in H4IIE cells. Furthermore, TNFα had no effect on the phosphorylation of Akt in response to insulin which indicates that IKKβ does not cause an insulin resistance in H4IIE cells.

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Cluster analysis of co-regulated mRNAs of rat islets exposed to low, intermediate and high glucose

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Background and Aims: Rat β-cell survival and function are best preserved by culture at 10 mM glucose (G10) and deteriorate after a few days at low (G2–G5) or high (G30) glucose. To investigate the mechanisms by which glucose affects the β-cell phenotype, we tested the effects of increasing glucose concentrations on rat islet gene mRNA levels.
Materials and Methods: Male Wistar rat islets were cultured 18 h in the presence of G2, G5, G10 or G30 after 1 wk preculture in RPMI medium containing G10 and 5 g/L BSA. Directly after culture, we measured acute glucose-induced insulin secretion (RIA) and islet gene mRNA levels (Affymetrix Rat Genome 230 2.0 oligonucleotide arrays+real-time RT-PCR for selected genes)(n=4). GCOS software (Affymetrix) was used to calculate match/mismatch probe signal intensities and assess differences in mRNA abundance, expressed as absolute signal $\log_2$ ratios (SLR). Self-Organizing Map cluster analysis (GeneCluster2) was used to categorize groups of mRNAs with similar glucose concentration-dependent changes in RNA abundance. The function of glucose co-regulated genes within each cluster was analysed with GenMapp 2.
Results: Islet insulin secretory response was best preserved after culture in G10, and was reduced by 50–75% without changes in glucose sensitivity after culture in G2–G5. Culture in G30 decreased both the threshold concentration of glucose activation and caused ~30% reduction in maximal glucose stimulation. In parallel to these functional changes, large scale changes were observed in the islet mRNA profile. From the 31099 probe sets on the arrays, 18081 were reliably detected in at least one glucose concentration. The SLR for at least one of the comparisons between the different glucose concentrations was≥2,≥1 and≥0.5 in 170, 1039 and 3530 probe sets respectively. Of the latter, 3120 were classified in 6 clusters with monophasic concentration-dependencies that differed in overall direction (increase/decrease) and glucose threshold. The remaining 410 probe sets were grouped into 4 small clusters with complex mRNA expression profiles (V-shaped or inverted V-shaped with minimum or maximum in G5 or G10). Analysis of gene function in each cluster suggests the presence of co-regulated metabolic and signaling pathways in rat islets. Thus, genes involved in cholesterol and unsaturated fatty acid synthesis were up-regulated between G2–G5 and G10–G30. In contrast, ATF4 target genes (integrated stress response) and other pro-apoptotic genes were down-regulated from G2–G5 to G10–G30. On the other hand, most glycolytic enzymes, XBP-1 target genes (unfolded protein response) and several proteasome subunits were mainly up-regulated between G10 and G30. Finally, several stress response genes had a V-shaped mRNA expression profile with a minimum in G10 and significant increases in both G2 and G30.
Conclusion: Assessment of the islet mRNA abundance after 18 h exposure to a large range of glucose concentrations reveals at least 10 distinct clusters of concentration-dependent co-regulated genes. We propose such level of analysis provides additional information to comparison of two glucose concentrations in a two-state model. Our results will help identifying key molecules that maintain the differentiated β-cell phenotype as well as those responsible for β-cell deterioration after exposure to chronic hypo- or hyperglycemia.

PS 26

Intracellular mediators of insulin secretion

0488

Extracellular ATP modulates insulin secretion via functionally active P2X and P2Y receptors

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Background and Aims: Extracellular ATP (eATP) modulates several cell functions via specific receptors, named P2X and P2Y on the basis of their molecular structure. The role of these receptors in insulin release has been little explored, and available results are controversial.
Materials and Methods: We measured insulin release by INS-1e cells grown at 11 mM glucose and incubated at 3.3, 7.5, 11 or 20 mM glucose for 60 min. After chronic (48 hours) challenge with different extracellular nucleotides (ATP, BzATP, a preferential $P2X_7$ agonist, and UTP, a preferential P2Y agonist), insulin release and total content

were measured by RIA. Moreover, P2 receptors (P2Rs) expression was aestimated by RT-PCR and Western blot, intracellular ATP (iATP) and eATP concentrations by luminometric assay, $[Ca^{2+}]_i$ by fluorimetry with the fluorescent indicator Fura2/AM, and apoptosis by FACS analysis.

Results: After exposure to 0.01–1.0 mM eATP, insulin secretion (expressed as ratio between release and content) was significantly reduced at all the glucose concentration tested (eg, with 0.5 mM ATP: from 5.3±0.1 to 1.9±0.2% at 3.3 mM glucose; from 8.6±0.1 to 4.7±0.1% at 7.5 mM; from 8.0±0.2 to 5.0±0.3% at 11 mM; from 11.5±0.2 to 7.4±0.4% at 20 mM). This inhibitory effect of eATP was fully prevented by either oxidized ATP (which blocks all P2X receptors) or apyrase (which hydrolyses ATP). In control incubations, eATP release increased with increasing glucose concentrations (37% and 58% above baseline with glucose 11 and 20 mM). BzATP, a preferential $P2X_7$ agonist, did not significantly influence insulin release (eg, with 0.5 mM BzATP: from 1.2±0.3 to 1.8±0.1% at 3.3 mM glucose; from 4.2±0.2 to 5.9±0.5% at 7.5 mM; from 6.2±0.7 to 6.8±0.4% at 11 mM; from 7.7±0.8 to 7.2±1.2% at 20 mM), while UTP, at concentrations between 0.01–0.1 mM, stimulated insulin release (eg, with 0.1 mM UTP: from 2.6±0.6 to 2.8±0.5% at 3.3 mM glucose; from 4.2±1.0 to 8.6±0.8% at 7.5 mM; from 7.8±1.3 to 9.3±0.9% at 11 mM; from 6.9±1.1 to 9.8±0.8% at 20 mM). A $[Ca^{2+}]_i$ rise after acute challenge with extracellular nucleotides was observed (Δ $[Ca^{2+}]_i$:180±36 nM after stimulation with ATP 1 mM); UTP was less effective. With regard to receptor(s) putatively involved in this process, both P2X (subtypes 1, 3, 4, 5, 6 and 7) and P2Y receptors (subtypes 1, 2, 4 and 6) were found to be expressed, but the level of expression was unchanged following nucleotide stimulation, except for $P2X_1$ and $P2X_7$ (the latter was present as a splicing form), whose expression was upregulated by ATP and BzATP, as shown by RT–PCR and WB. Neither early nor late apoptosis was enhanced by nucleotide stimulation (early apoptosis: after 3 hrs: annexin–propidium positivity: from 1.30 to 1.10 with UTP 0.1 mM, to 1.71 with BzATP 0.5 mM, to 1.86% with ATP 0.5 mM; after 24 hrs: from 1.09 to 1.81 with UTP 0.1 mM, to 1.20 with BzATP 0.5 mM, to 0.92% with ATP 0.5 mM; late apoptosis: after 24 hrs: hypodiploid peak lower than 1% in both basal and stimulated cells).

Conclusion: In INS-1e cells pharmacologic P2X receptor stimulation downregulates, and P2Y receptor stimulation upregulates, insulin synthesis and release. This effect could be intrinsically modulated by the enhanced eATP released by INS-1e cells in the presence of increasing glucose concentrations. Neither effect is explained by changes in apoptosis. $P2X_7$ expression was enhanced by ATP, but this was not coupled with changes in insulin release, suggesting that $P2X_7$ receptors in these cells may only be involved in cytokine release.

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0489

Involvement of Src in signal-transducing function of Na^+/K^+ ATPase to decrease ATP production by generating reactive oxygen species in pancreatic islets

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Background and Aims: Na^+/K^+ ATPase inhibition by ouabain suppresses mitochondrial ATP production by generating reactive oxygen species (ROS) that accounts for the late suppressive effect (after 30 min) on glucose-induced insulin secretion from pancreatic islets. As ouabain also suppresses the ATP level by generating ROS in the Ca^{2+}-deprived condition in pancreatic islets, the signal-transducing function of Na^+/K^+ ATPase is Ca^{2+}-independent. Ouabain causes a rapid activation of Src that results in ROS generation in cardiomyocytes. To clarify the signal-transducing function of Na^+/K^+ ATPase to decrease ATP production by generating ROS in pancreatic islets, the involvement of Src was examined.

Materials and Methods: After isolated islets from Wistar rats were cultured with RPMI medium containing a normal level of Ca^{2+}, experiments were performed in Ca^{2+}-deprived condition. ATP content was determined by luminometric method. ROS was measured by CM-DCF fluorescence using dispersed islet cells. After lysates were immunoprecipitated by anti-Src antibody, immunoblotting was performed using anti-Src, anti-Src[pY418], and anti-Src[pY529] antibodies.

Results: Ouabain (1 mM) caused a rapid activation of Src in the presence of 16.7 mM glucose. Maximum increase in Tyr^{418} phospholyration, which indicates activation of Src, was observed 3 min after ouabain exposure. Ouabain increased Tyr^{418} phospholyration but did not alter Tyr^{529} phospholyration, indicating inactivation of Src (fold increase relative to control at 3 min, pY418: 1.79±0.15, $P<0.01$; pY529: 1.09±0.04, not significant; total Src: 0.97±0.03, not significant). Ouabain suppressed the glucose-induced increase in ATP content at 60 min (2.8 mM glucose, 9.1±0.5; 16.7 mM glucose, 17.9±0.7; 16.7 mM glucose plus ouabain, 12.3±0.5 pmol/islet, $P<0.01$ vs.

16.7 mM glucose alone). However, in the presence of 10 μM PP2, a specific Src inhibitor, ouabain did not affect ATP contents (16.7 mM glucose, 16.2±0.9; 16.7 mM glucose plus ouabain, 17.2±0.9 pmol/islet, not significant). The abolished decrease in ATP content by ouabain also was observed in the presence of other Src inhibitors including herbimycin A and SU6656. Ouabain increased ROS production in the presence of 16.7 mM glucose (at 60 min, 100±4, control; vs. 128±6% of control, ouabain, P=0.01). PP2 decreased ouabain-induced ROS production (ouabain plus PP2, 103±5% of control, P=0.02 vs. ouabain).

Conclusion: Na^+/K^+ ATPase inhibition by ouabain suppresses ATP content and increases ROS production by activating Src. Thus, Src plays a role in the signal-transducing function of Na^+/K^+ ATPase to decrease ATP production by generating ROS in pancreatic islets.

0490

L-Cysteine inhibits insulin release through the metabolic production of hydrogen sulfide, a novel gasotransmitter

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Background and Aims: L-Cysteine is a sulphur-containing amino acid distributed in a wide variety of tissues. Recent studies demonstrated that hydrogen sulfide (H_2S), a metabolite of L-cysteine, acts as an intracellular gasotransmitter in various types of cells including neural cells and smooth muscle cells. Moreover, increases of the L-cysteine contents have been reported in plasma and some tissues in diabetic subjects or model animals. Nevertheless, the roles of H_2S in the endocrine tissues remain unrevealed. The aim of the present study is to investigate the effect of L-cysteine and H_2S on insulin release from the mouse pancreatic β-cells.

Materials and Methods: Pancreatic islets were isolated from male ICR mice aged 8–12 weeks by the collagenase digestion method. Expression of cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CSE), both of which produce H_2S from L-cysteine, was determined by RT-PCR and immunoblot analysis. Insulin release from isolated islets was examined by batch incubation and insulin released into the media was measured by radioimmunoassay. For $[Ca^{2+}]_i$ measurement, pancreatic islets were dispersed by dispase and preloaded with the Ca^{2+}-sensitive fluorescent dye, fura PE3. Real-time imaging of $[Ca^{2+}]_i$ was carried out by a dual-wavelength microfluorometry system. The ATP content in the β-cell line, MIN6 cells, was measured by the luciferin-luciferase reaction and the mitochondrial membrane potential ($\Delta\Psi_m$) in a single β-cell was monitored with rhodamine 123, a fluorescent indicator of $\Delta\Psi_m$.

Results: We found CBS and CSE were expressed in mouse pancreatic islets and MIN6 cells. L-cysteine and the H_2S donor NaHS inhibited glucose-induced insulin release from islets and MIN6 cells in a dose-dependent manner. These inhibitory effects were reproduced when insulin release was stimulated by α-ketoisocaproate, tolbutamide or high K^+. Both substances suppressed ATP production and attenuated glucose-induced hyperpolarization of the β-cell $\Delta\Psi_m$. These substances promptly and reversibly suppressed glucose-induced $[Ca^{2+}]_i$ oscillation in a single β-cell without obvious changes in the mean $[Ca^{2+}]_i$ value, suggesting that L-cysteine and NaHS inhibit insulin release via their opening effects on the K_{ATP} channels and resultant $[Ca^{2+}]_i$ changes. In the presence of diazoxide, however, L-cysteine and NaHS still inhibited high K^+-induced insulin release. Furthermore, both remained effective to inhibit Ca^{2+}-induced insulin release when the islet cell membranes were permeabilized with streptolysin-O. Therefore, we suppose the inhibition of insulin release includes K_{ATP}-independent mechanisms.

Conclusion: H_2S produced by L-cysteine metabolism potently inhibits insulin release via multiple actions on the insulin secretory machinery. We suppose that increased L-cysteine contents and eventual H_2S production under the diabetic condition may affect the β-cell functions and that it may be involved in the deterioration of diabetes mellitus.

0491

Intracellular GABA accumulation suppresses glucose-induced insulin secretion in rat pancreatic islets as well as membrane depolarisation in β cells

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Background and Aims: Overexpression of GAD65 in mice (Shi Y. et al, Am. J. Physiol. Endocrinol. Metab. 279, E684, 2000) or INS-1 cells (Rubí B. et al., J. Biol. Chem. 276, 36391, 2001) diminishes their secretory response to glucose. We have investigated whether increased intracellular GABA concentrations affect the mechanism of stimulation of insulin secretion in rat pancreatic islets and isolated β-cells.

Materials and Methods: Islet α-amino acids were measured fluorometrically after precolumn derivatization with o-phtalaldehyde and HPLC separation, glucose utilization was measured as the amount of ^3H_2O-production from 20 mM D[5-^{3}H]glucose, ATP/ADP with the luciferase/

luciferin sytem, and insulin was radioimmunologically measured in batch-type incubations (1 hour). Membrane potential and whole-cell K_{ATP} currents in isolated β-cells were measured in the perforated patch whole-cell configuration of the patch-clamp technique, while single K_{ATP}-channel currents were recorded from inside-out or outside-out membrane patches.

Results: Islet incubations with a range of ethyl-GABA (eGABA, a membrane permeable GABA precursor) concentrations (0.25,1, 5, and 10.0 mM) induced a linear increase of the islet GABA content that exceeded by 2- (at 5 mM eGABA) and 4-fold (at 10 mM eGABA) the level obtained at 1 mM L-glutamine. No significant variation was observed in other islet α-amino acids. 5 mM eGABA suppressed by more than 50% the islet secretory response to 20 mM glucose (30.8±3.7, n=5, vs. 65.8±3.3 pg/min x islet, n=12; p<0.0001). 10 mM eGABA decreased the rate of 20 mM glucose utilization within 22 to 37% (with or without pre-exposure to eGABA, respectively) as well as the rate of oxidation (17.9±0.6, n=5; vs. 24.5±0.9 pmol/h x μg protein, n=6; p<0.001) and the islet content of ATP (4.1±0.1, n=6, vs. 4.91±02 pmol/islet, n=6; p<0.01). In the presence of 10 mM glucose, 5 mM eGABA reversibly hyperpolarised isolated β-cells from −19±3 mV to −49±4 mV (n=6; p<0.001) and increased the whole-cell resting conductance by 213±127% (p<0.01). These effects were also completely reversed by tolbutamide (100 μM, n=5). Extracellular application of GABA (5 mM) had no effect on β-cell membrane potential or conductance (n=3). Bath application of eGABA (5 mM) did not activate K_{ATP} single channel currents in isolated inside-out (n=3) or outside-out (n=7) membrane patches.

Conclusion: 1) An increase of the intracellular GABA concentration above physiological values decreased insulin secretion in response to glucose with higher efficiency than islet metabolism. 2) eGABA reversed glucose-induced β-cell depolarization by increasing ATP-dependent K^+-channel activity. 3) It is hypothesized that GABA uptake into the synaptic like microvesicles of β-cells might compete for cytosolic ATP with the K^+-channels.

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Inhibitors of phospholipase A2 rapidly suppresses glucose-induced Ca^{2+} oscillations in pancreatic β cells

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Background and Aims: Phospholipase A2 (PLA_2) generates arachidonic acid (AA) and lysophospholipids. Pancreatic β-cells express both cytoplasmic $cPLA_2\alpha$ activated by Ca^{2+} and $iPLA_2$ activated by internal ATP. External ATP is a stimulator of $cPLA_2$ in isolated mouse islets and AA has a suppressive action on KATP channels both in insulin-secreting HIT cells and in mouse β-cells. A role for AA in glucose-induced rhythmicity of cytoplasmic Ca^{2+} is suggested from observations that different external activators of $cPLA_2$ induce Ca^{2+} oscillations. These observations, together with a report that AA induces Ca^{2+} oscillations in the exocrine pancreas, gave rise to the question whether AA participates in the generation of the Ca^{2+} oscillations responsible for pulsatile insulin release.

Materials and Methods: The cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]i$) was studied in single mouse β-cells (Umea ob/ob), using ratiometric fura-2 technique. Measurements were performed with an inverted microscope equipped for epifluorescence. The cells were illuminated only during the image capture and the excitation light was kept at a minimum. After raising the glucose concentration from 3 to 20 mM the β-cells were exposed to the PLA_2 inhibitors N-(p-amylcinnamoyl) anthranilic acid (ACA), arachidonyl trifluoromethyl ketone ($AACOCF_3$) and bromoenol lactone (BEL). For comparative purposes suppression of the AA formation with the diacylglycerol lipase inhibitor:1,6-bis-(cyclohexyl-oximinocarbonylamino)-hexane (RHC 80267) was tested.

Results: Oscillations of $[Ca^{2+}]i$ from a basal level were seen in most β-cells superfused with 20 mM glucose. ACA at 25 μM had often a suppressive action and 35 μM was sufficient to remove the $[Ca^{2+}]i$ oscillations in the β-cells. The action of ACA was reversible. Reappearance of oscillations in the presence of ACA was occasionally seen after addition of 100 μM tolbutamide but not in the presence of 0.1 μM BAY K 8644 or 0.1 μM ATP. $AACOCF_3$ (10 μM) effectively removed the oscillations of $[Ca^{2+}]i$. However, omission of 25–35 μM of this $cPLA_2$ inhibitor did not result in a reappearance of the oscillations. The oscillations became lower and started from an elevated level at 10 μM BEL. At a concentration of 15 μM, BEL induced complete loss of the oscillations, which often reappeared after omission of the inhibitor. The diacylglycerol lipase inhibitor RHC (5–10 μM) effectively removed the $[Ca^{2+}]i$ oscillations, an effect reversed after omission of the substance or addition of 100 μM tolbutamide.

Conclusion: Both low concentrations of inhibitors of PLA_2 and suppression of diacylglycerol lipase remove the $[Ca^{2+}]i$ oscillations in pancreatic β-cells. The results support the idea that AA is important for the $[Ca^{2+}]i$ rhythmicity responsible for pulsatile release of insulin.

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Effects of glucose and tolbutamide on mitochondrial Ca^{2+} handling of insulin secreting cells

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Background and Aims: Cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) and mitochondrial function of pancreatic B-cells are tightly coupled. Increases in $[Ca^{2+}]_c$ in response to glucose reduce mitochondrial ATP production thus contributing to a negative feedback mechanism which regulates B-cell oscillatory activity. Since mitochondrial Ca^{2+} ($[Ca^{2+}]_m$) was suggested to be the determinant for oscillatory ATP production, experiments were performed to evaluate whether $[Ca^{2+}]_m$ also fluctuates. Sulfonylureas are known to increase Ca^{2+} influx across the plasma membrane. We examined in this study whether sulfonylureas also interfere with B-cell Ca^{2+} homeostasis by affecting Ca^{2+} storage into mitochondria.
Materials and Methods: Experiments were carried out with B-cells from C57BL/6 mice or INS-1 cells using fluorescence techniques. $[Ca^{2+}]_c$ was determined by fura-2. Full width at half maximum of the Ca^{2+} peak ($width_{0.5P}$) was used as an indicator for the amount of Ca^{2+} released from intracellular stores. $[Ca^{2+}]_m$ was estimated by ratiometric pericam targeted to mitochondria. INS-1 cells were transfected with the plasmid pcDNA3 by effectene.
Results: Increasing the glucose concentration from 0.5 to 15 mM induced oscillations in $[Ca^{2+}]_m$ in INS-1 cells with a mean maximal amplitude of 56±3 a.u. (arbitrary units, n=7) supporting the hypothesis that $[Ca^{2+}]_m$ exhibits the link between $[Ca^{2+}]_c$ and fluctuations in mitochondrial metabolism. In the presence of 15 mM glucose FCCP (0.5 μM) induced a transient increase of Ca^{2+} in B-cells from 355±39 nM to 606±74 nM ($width_{0.5P}$ 32±4 s, n=6). This release was completely suppressed by SERCA inhibition with cyclopiazonic acid (CPA, 10 μM) (n=7). In the presence of tolbutamide (1 mM) FCCP led to enhanced Ca^{2+} release ($width_{0.5P}$ 89±10 s, n=5) that even persisted with CPA ($width_{0.5P}$ 35±7 s, n=4). This observation suggests that Ca^{2+} is stored in non-ER Ca^{2+} pools as a consequence of sulfonylurea treatment. Indeed tolbutamide enhanced $[Ca^{2+}]_m$ to 271±59 a.u. (n=6) which was about 5fold larger than the increase observed with 15 mM glucose ($P \leq 0.002$). This suggests that the non-ER Ca^{2+} stores recruited by tolbutamide are the mitochondria.
Conclusion: $[Ca^{2+}]_m$ is able to oscillate in response to glucose. We conclude that these oscillations trigger fluctuations of the mitochondrial membrane potential $\Delta\Psi$ and thus ATP production. It is demonstated that sulfonylureas influence mitochondrial Ca^{2+} handling. Compared to control cells where the ER is the predominent Ca^{2+} store Ca^{2+} uptake into mitochondria gains in importance in the presence of sulfonylureas. The latter effect may be involved in Ca^{2+}-dependent apoptosis induced by sulfonylureas.
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0494

K_{ATP} channel activity in pancreatic beta cells is regulated by an adenylate kinase

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Background and Aims: The cytosolic ATP concentration $[ATP]_c$ of pancreatic B-cells is about 30fold higher than necessary for complete inhibition of K_{ATP} channels in cell-free patches. Nevertheless, in intact cells insulin secretion in response to glucose is regulated by changes in K_{ATP} channel activity. It is known from cardiac myocytes that a metabolic barrier insulates K_{ATP} channels from $[ATP]_c$. We suggest that an adenylate kinase (AK) may be involved in the regulation of K_{ATP} channel activity in B-cells. This enzyme can form ADP in the presence of ATP and AMP and thus counterbalance channel inhibition by ATP.
Materials and Methods: Experiments were carried out with B-cells from C57BL/6 mice. Single K_{ATP} channel current measurements were performed in the open cell attached (oca) or inside/out (i/o) mode of the patch-clamp technique. The AK isoform of B-cells was identified by Western blots and immuno staining experiments.
Results: ATP concentration response curves show a clear left-shift in the open probability (NPo) of K_{ATP} channels when i/o currents were compared to oca measurements (Km=6.6±0.7 μM and 27.5±14.8 μM, respectively), pointing at an influence of cytosolic components on the ATP block. Accordingly, NPo of K_{ATP} channels in the oca patch in the presence of 0.125 mM ATP was increased by 595±107% (n=5) by addition of 0.1 mM AMP. The same manoeuvre was without effect in i/o patches (n=5) or in oca patches where AK was inhibited by Ap_5A (20 μM, n=6). Western blot analysis of whole islets exhibited a positive response for the AK isoform 1 (AK1). This was confirmed by immunofluorescence images showing a co-localization of AK1 with insulin.
Conclusions: We conclude that an adenylate kinase, probably AK1, is part of a metabolic barrier shielding

K_{ATP} channels of pancreatic B-cells from the $[ATP]_c$ and thus allowing opening of K_{ATP} channels despite the extremely high bulk ATP concentration.
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0495

Role of the Na:H exchanger in β cell stimulus-secretion coupling: investigation in mice with a spontaneous inactivating mutation

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Background and Aims: Pharmacological studies have suggested that both Na:H and Cl: HCO_3 exchangers regulate cytosolic pH (pH_i) in β-cells, but their respective role has not been defined. This complicates the appraisal of models implicating pH_i changes in stimulus-secretion coupling. Thus, the mechanisms underlying these changes and their possible influence on exocytosis of insulin granules remain unclear. Having identified NHE1 as the major isoform of the Na:H exchanger in islets, we used mice bearing an inactivating mutation of NHE1 (*Slc9A1 – swe/swe*) (Swe mice) (Cell 91: 139–148, 1997) to investigate the role of Na:H exchange in β-cell function.
Methods: Islets were isolated from genotypically identified, homozygous Swe mice of 6–8 weeks, as these animals do not live much longer because of ataxia and seizures. The islets were cultured for 1 day before measurement of insulin release, pH_i and cytosolic Ca^{2+} ($[Ca^{2+}]_i$) (BCECF and FURA-P3 methods). Experiments were performed in HCO_3-buffer (gassed with O_2–CO_2) and HCO_3-free, Hepes-buffer (gassed with O_2), conditions under which both Cl:HCO_3 and Na:H exchangers or only Na:H exchanger can operate.
Results: Compared to age-matched controls, the body weight of Swe mice was 35% smaller, their islet insulin content was 30% lower, but their blood glucose was not different. Basal pH_i was lower in Swe islets (~6.7) than control islets (~6.9) in Hepes-buffer but not in HCO_3-buffer. The ability of the islets to regulate pH_i was tested by the NH_4Cl pulse technique. In HCO_3-buffer, Swe islets corrected the acidification slightly more slowly than controls whereas, unlike controls, they failed to correct the acidification in Hepes-buffer. These results indicate partial redundancy of the two exchangers in controls and absence of pH_i regulators other than Cl: HCO_3 exchangers in Swe islets. In HCO_3-buffer, stimulation with 15 mmol/l glucose caused a similar small increase in pH_i, rise in $[Ca^{2+}]_i$ with oscillations in the steady-state, and biphasic release of insulin in control and Swe islets. After correction for differences in islet insulin content, the rate of insulin release was even higher in Swe islets. Stimulation with 30 mmol/l KCl produced similar effects in both types of islets: a small decrease in pH_i and large increases in $[Ca^{2+}]_i$ and insulin release. In Hepes-buffer, the behaviour of control and Swe islets was different. In controls, 15 mmol/l glucose now slightly lowered pH_i while increasing $[Ca^{2+}]_i$ and insulin release as in HCO_3-buffer. In contrast, a marked decrease in pH_i occurred in glucose-stimulated Swe islets, accompanied by a progressive decrease in the insulin secretory rate. Similar alterations of pH_i and insulin release were observed when Swe islets were stimulated with KCl in Hepes-buffer.
Conclusions: Loss of Na:H exchange has no significant consequence on stimulus-secretion coupling in mouse β-cells as long as islets are maintained in physiological HCO_3-buffer. A negative impact is only detected in Hepes-buffer, when Cl:HCO_3 exchangers cannot operate and pH_i falls to very low values that are not normally observed. The results also suggest that the small changes in pH_i occurring in control islets under physiological conditions are unlikely to influence insulin release directly.

0496

Expression and role of sodium pump in insulin secreting cells

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Background and Aims: Stimulation of insulin secretion by Ca^{2+}-influx depends on changes of membrane potential. The Na^+/K^+-ATPase creates the potassium gradient over the plasma membrane, the main gradient responsible for the resting membrane potential in insulin secreting cells. Due to the electrogenic nature of the Na^+/K^+-ATPase, stimulation of the enzyme generates a hyperpolarizing current, which may contribute to the stabilisation of the resting membrane potential and retarding Ca^{2+} entry. The aim of the present study was to examine the molecular composition of Na^+/K^+-ATPase in insulin secreting cells and to analyse whether glucocorticoids which are known to inhibit insulin secretion alter its expression and activity.
Materials and Methods: Semiquantitative real time RT/PCR was used to quantify mRNA levels of Na^+/K^+-ATPase subunits in insulin secreting INS-1 cells. Pump activity was estimated by the mean of outward current generated by the enzyme when KCl was added to an extracellular solution devoid in potassium using the whole cell configuration of the patch clamp method.

Results: INS-1 cells express α_1-, β_1- and β_3-subunits of rat Na^+/K^+-ATPase but not α_2, α_3 and β_2. Cell culture with 100 nM dexamethasone for 1 day increased the mRNA levels of α_1- and β_1- subunits six- and twofold, respectively. The addition of 5 mM KCl to a bath solution lacking potassium initiated an outward current of 0.55±0.02 pA/pF (n=26) in INS-1 cells and of 0.77±0.17 pA/pF (n=5) in isolated mouse islet cells. INS-1 cells were hyperpolarized by −16.5±2.7 mV, mouse islet cells by −15.7±3.1 mV. Ouabain (1 mM) or omission of ATP from the pipette solution almost completely inhibited the effects of KCl. Pretreatment of INS-1 cells with 100 nM dexamethasone for 1 day increased outward current significantly by 25% to 0.72±0.05 pA/pF (n=15), an effect which was antagonized by the glucocorticoid receptor antagonist RU486, 1 μM.
Conclusion: Insulin secreting INS-1 cells express the α_1-, β_1- and β_3-subunits of Na^+/K^+-ATPase. The increased expression and pump activity observed after dexamethasone may stabilize the resting membrane potential and thus contribute to the known reduction of glucose-induced insulin secretion after glucocorticoid excess.

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Mediators of insulin secretion

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Functional heterogeneity in pancreatic beta cell subpopulations in Wistar rats

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Background and Aims: The sialylated form of the neural cell adhesion molecule (PSA-NCAM) which is present on the pancreatic beta-cell membrane is a good tool to sort different beta-cell subpopulations according to their functional activity. We previously showed a close correlation between abundance of PSA-NCAM and insulin response to glucose: high PSA-NCAM-labelled beta-cells (beta$^+$-cells) secreted much more insulin in response to glucose than low PSA-NCAM-labelled beta-cells (beta$^-$cells). The present study compared some aspects of the intracellular mechanisms that could explain this differential response to glucose in these two beta-cell subpopulations. For this purpose, we examined the extent to which the differences in the glucose-stimulated insulin secretion in the beta$^+$ and beta$^-$cells was related 1/to the handling of cytoplasmic Ca^{2+} concentrations and 2/to the cAMP cellular levels in consideration of the role of cAMP in glucose-induced insulin secretion. In addition, because responsiveness might be variable according to the nature of the stimulus, we tested whether this different functional activity between the two subpopulations is glucose-specific or not.
Materials and Methods: Islet cells suspensions from Wistar rats were sorted by flow cytometry, according to their PSA-NCAM abundance using an anti-PSA-NCAM antibody. Insulin release was measured in sorted beta-cell subpopulations by a perifusion technique in a system allowing concomitant recording of intracytoplasmic Ca^{2+} movements ($[Ca^{2+}]i$) by microfluorimetry. Using batch-incubated cells, cAMP content and insulin release in response to glucose (5.5 and 16.7 mM) and/or 10 nM GLP-1, 19 mM arginine, 10 mM leucine were studied.
Results: In beta$^+$-cells, insulin release, in response to 16.7 mM glucose, was highly increased compared to beta$^-$cells and was correlated with an increased in $[Ca^{2+}]i$. cAMP content, measured in presence of 16.7 mM glucose without or with GLP-1 was increased compared to 5.5 mM glucose. In parallel, insulin secretion in response to 16.7 mM glucose was potentiated by GLP-1. By contrast, in beta$^-$cells, insulin release and $[Ca^{2+}]i$ variations, in response to glucose 16.7 mM were weak compared to beta$^+$-cells. Furthermore, beta$^-$cells were not responsive to 16.7 glucose alone or glucose+GLP-1. Accordingly, their cAMP content was broken down. However, addition of KCl (50 mM), at the end of perifusion experiments showed that beta$^-$cells were capable to secrete insulin to the same extent as beta$^+$-cells. Moreover non-glucose secretagogues, arginine and leucine elicited the same high insulin release in both subpopulations.
Conclusion: Alteration of the functionality of beta cells could be explained by a blockage in the step(s) linking glucose metabolism to the insulin release process. The analysis of glucose-stimulated insulin release characteristics in beta-cell subpopulations with a different secretory activity is a prerequisite to identify factors able to ameliorate the functional status of beta-cells with low secretory capacities such as in advancing type 2 diabetes.

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The direct effects of exendin-4 on human non-diabetic and type 2 diabetic islets

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Background and Aims: Exendin-4, a long-acting GLP-1 mimetic, has several beneficial metabolic effects, and its

synthetic analogue Exenatide has been proposed in the therapy of Type 2 diabetes. However, little information is available on the direct actions of Ex-4 on human pancreatic islets (HI), in particular in the case of diabetes.

Materials and Methods: Islets were prepared from the pancreas of 18 multiorgan donors (11 non-diabetic, ND: 6F/5M; age, 66±19 yrs; BMI, 24.5±3.3 kg/m2; and 7 with Type 2 diabetes, T2D: 5F/2M; age, 63±9 yrs; BMI: 27.4±4.5 kg/m2). Then dose-response (Ex-4 from 1 to 100 nmol/l) and time-response (incubation from 1 to 7 days) experiments were performed, and 10 nmol/l Ex-4 and 2 and 7 days of incubations were further investigated.

Results: Glucose-stimulated insulin secretion improved significantly ($p<0.05$) after incubation with 10 nmol/l Ex-4 (stimulation index from 2.1±0.3 to 2.55±0.25 in ND and 1.27±0.3 to 1.96±0.4 in T2D). In both ND and T2D islets Ex-4 induced a significantly ($p<0.05$ or less) higher gene expression (by quantitative Real-Time RT-PCR) of insulin (respectively+93±38% and+37.5±17.0%), GLUT2 (+283±41% and+178.6±75.9%), glucokinase (+117±45% and+80.8±16.1%), PDX-1 (+470±149% and+157.5±78.0%) and Ki67 (+217±55% and+355.0±78.2%) after 2 days of incubation, which was maintained at 7 days. Furthermore, in ND Ex-4 caused a significantly ($p<0.05$ or less) increased transcription of several additional genes, including PAX4 (+115±27%), NKX2.2 (+45±33%) and NKX6.1 (+176±22%), which, however, was not observed in T2D.

Conclusions: In conclusion, Ex-4 has beneficial effects on isolated human non-diabetic islets; most importantly, part of these positive actions are exerted also on Type 2 diabetic islets, supporting the concept that incretins are useful tool in the therapy of diabetes.

0499

Combined effects of exenatide and glibenclamide on insulin secretion by the perfused rat pancreas

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Background and Aims: Exenatide (exendin-4, Ex-4) is an incretin mimetic exerting glucose-dependent enhancement of insulin secretion similar to that of glucagon-like peptide 1. Ex-4 treatment has been shown to reduce HbA_{IC} in type 2 diabetic patients unable to achieve adequate glycemic control under maximally effective doses of metformin and/or a sulfonylurea. In this work we have investigated the effects of 1 nM Ex-4 and 0.1 μg/ml glybenclamide (Glyb), alone and in combination, on insulin secretion.

Materials and Methods: The study was performed in the perfused rat pancreas. Experiments were performed at high (9 mM), normal (5.5 mM) and low (3.2 mM) glucose levels.

Results: At 9 mM glucose, the insulin response to Ex-4 (1175±171, SEM, ng/15 min) was greater than that to Glyb (596±62 ng/15 min; $p<0.01$). When Ex-4 and Glyb were coinfused, an additive effect on insulin secretion was observed (1681±363 ng/15 min). At 5.5 mM glucose, the insulin responses to Ex-4 (128±39 ng/15 min) and to Glyb (215±39 ng/15 min) were not significantly different. The insulin secretion induced by Ex-4+Glyb (1141±199 ng/15 min) was greater than the sum of the individual responses to these two secretagogues, thus indicating a synergistic effect on the B cell. At 3.2 mM glucose, Ex-4 did not affect basal insulin levels (1.2±1.5 ng/15 min) while Glyb stimulated insulin secretion (143±44 ng/15 min). Coinfusion of Ex-4 markedly potentiated the insulinotropic effect of Glyb (435±85 ng/15 min; $p<0.025$ vs. Glyb experiments).

Conclusion: Our findings confirm the glucose-dependent insulinotropic effect of Ex-4. Concerning the combined effects of Ex-4 and Glyb on insulin secretion, the synergism observed at normal (5.5 mM) glucose levels would indicate that Ex-4 may exert a glucose-like priming effect of the B cell. Consequently, in the presence of Ex-4, sulfonylurea may induce an insulinotropic effect comparable to that found at high glucose concentration. In the isolated rat pancreas, this priming effect becomes more evident at low glucose levels as Ex-4 renders the B cell competent to the insulinotropic effect of Glyb. Importantly, our "*in vitro*" observations must be taken in context, as in the intact animal/human, incretin-mediated glucose-dependent stimulation of insulin is tightly regulated by multiple extra-pancreatic (e.g. CNS) feedback control systems that would not exhibit any influence in the isolated preparation used in the present study.

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0500

Ghrelin attenuates glucose-induced insulin release in an autocrine/paracrine manner in pancreatic islets

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Background and Aims: Ghrelin, isolated from the human and rat stomach, is the endogenous ligand for the growth

hormone secretagogue receptor (GHS-R). We have reported that ghrelin and GHS-R were expressed in pancreatic islets and that ghrelin suppressed glucose-induced insulin release via activation of voltage-dependent delayed rectifier K^+ channels and attenuation of glucose-induced action potentials, which leads to suppression of glucose-induced Ca^{2+} signaling in β-cells. In this study, we aimed to examine (1) whether ghrelin is released from islet cells and (2) whether ghrelin in islets seves as a physiological regulator of insulin release.

Materials and Methods: To measure plasma ghrelin concentrations, blood samples were collected from the pancreatic arteries (celiac artery) and veins (splenic vein), and portal veins of anaesthetized rats or mice. Plasma concentrations of acylated- and desacyl-ghrelin were measured using enzymeimmunoassay. Ghrelin release from single islet cells were measured using single cell immunoblot assay. Insulin release was measured in rat perfused pancreas and isolated islets prepared by collagenase digestion, and determined using radioimmunoassay and enzymeimmunoassay, respectively.

Results: Plasma acylated- and desacyl-ghrelin concentrations were higher in pancreatic vein than in artery in rats. Single cell immunoblot analysis revealed ghrelin secretion from islet β- and α-cells. Both GHS-R blockade by a GHS-R antagonist [D-Lys^3]-GHRP-6 and ghrelin immunoneutralization with anti-ghrelin antiserum increased glucose (8.3 mM)-induced insulin release in rat perfused pancreas and isolated islets. Glucose-induced insulin release from isolated islets of ghrelin knockout (Ghr-KO) mice was significantly greater than that of wild-type control mice, while insulin content was unaltered. Ghr-KO mice displayed increased insulin and decreased glucose responses in glucose tolerance tests. The profiles of insulin tolerance test exhibited no significant differences between Ghr-KO and control mice.

Conclusion: Ghrelin is released at least from islet β- and α-cells and it serves as a physiological autocrine/paracrine regulator to downregulate glucose-induced insulin release in islets.

0501

Stimulation of insulin release in MIN6 cells and isolated rodent islets by a small molecule glucokinase activator (GKA50)

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Background and Aims: Glucokinase (GK) is the glucose sensor in the β-cell and acts to control the threshold for glucose-stimulated insulin secretion (GSIS). Several small molecule direct activators of GK have been discovered which increase GSIS in β-cells and stimulate hepatic glucose metabolism. We have previously reported on a series of activators of human GK which lower plasma glucose levels after oral glucose tolerance test in female high fat fed Zucker rats and are a potential treatment for Type 2 diabetes.

The aims here were to determine the effects of one of these compounds, GKA50, on pancreatic GK activity and GSIS in mouse insulinoma cells (MIN6) and in isolated rodent islets (rat and mouse). GK inhibitors were used in MIN6 cells to demonstrate that the effects of GKA50 on insulin secretion were due to activation of GK.

Materials and Methods: Recombinant human and rat pancreatic GK were cloned from cDNA by PCR, expressed in E. coli with a 6-His tag and assayed for glucose phosphorylation spectophotometrically. MIN6 cells were cultured under standard conditions in DMEM with 25 mM glucose. Insulin release was measured by ELISA in media from 1 h incubations with 0–5 mM glucose and 1 nM –30 μM GKA50. The effects of the GK inhibitors, mannoheptulose and 5-thioglucose (10 mM), on insulin secretion were determined at 5 mM glucose and 10 μM GKA50 and compared with their effects on tolbutamide-stimulated (50 μM) insulin release. Islets were prepared from male Wistar rats by Liberase digestion and insulin secretion measured by comparison of release at basal (3 & 5 mM) and stimulated (8 & 10 mM) glucose concentrations after 2 h in static incubations. Islets were prepared from male C57Bl/6 mice by a similar method with insulin release measured at 3, 10 & 15 mM glucose.

Results: GKA50 activated recombinant human and rat pancreatic GK with EC_{50} values of 0.039 μM (n=10) and 0.059 μM (n=5) respectively. The mechanism of activation in rat and human enzyme was a clear concentration-dependent leftward shift in $S_{0.5}$ for glucose. In MIN6 cells at 2, 3 and 5 mM glucose GKA50 increased insulin release with EC_{50} values of 7.2 μM (n=5), 2.5 μM (n=3) and 0.84 μM (n=5) respectively. The GK inhibitors, mannoheptulose and 5-thioglucose, completely blocked the effects of GKA50 on insulin secretion in MIN6 cells at 5 mM glucose, but not those of tolbutamide. In Wistar rat islets incubated with 5 mM glucose, 1 μM GKA50 caused a 2.5 fold (n=5) increase in insulin release and 10 μM GKA50 caused a 4.4 fold (n=4) increase in insulin release. At 8 mM glucose GKA50 stimulated insulin release but this was not concentration-dependent. At 10 mM glucose there was no effect of GKA50 on insulin secretion. GKA50 was shown to stimulate insulin secretion of mouse islets in the presence of 10 mM glucose.

Conclusion: GKA50 concentration-dependently and glucose-dependently modulated insulin release from rat islets and MIN6 cells. Kinetically this represented a leftward shift in $S_{0.5}$ for glucose. Effects on insulin secretion from MIN6 cells can be completely blocked by GK inhibitors, entirely consistent with GKA50 being a specific glucokinase activator.

0502

Palmitic acid potentiates glucose induced insulin release in mouse islets via the GPR40 receptor

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Background and Aims: GPR40 is a GPCR that is preferentially expressed in the pancreatic β-cells. Medium and long chain fatty acids have been shown to stimulate insulin secretion through the activation of GPR40. GPR40 null mice are protected against the detrimental effects of a high fat diet and have improved glucose tolerance compared with wild type mice fed a high fat diet; whilst overexpression of GPR40 in the β-cells of mice leads to impaired β-cell function, hypoinsulinaemia and diabetes. These data suggest that chronic fatty acid stimulation of insulin secretion is detrimental and that a GPR40 antagonist would be beneficial in the chronic treatment of type 2 diabetes. The aims of this study were two-fold. Firstly, to test whether fatty acid potentiation of glucose induced insulin secretion is reduced in islets from GPR40 null mice and secondly, to investigate whether a small molecule GPR40 antagonist is able to inhibit fatty acid stimulation of glucose induced insulin secretion.
Materials and Methods: Pancreatic islets were isolated from C57/BL6J mice and GPR40 null and wild type mice using collagenase digestion. Glucose induced insulin secretion was measured in a static assay, with 3 islets per well in a 96 well plate, at high (16.7–20 mM) glucose. Palmitic acid was pre-conjugated to bovine serum albumin at a ratio of 5:1. Following two hours incubation with the specified reagents insulin was measured using an ELISA assay.
Results: 0.5 mM palmitic acid robustly (>2-fold) potentiated glucose induced insulin secretion in islets from wild type mice but not in islets from GPR40 null mice. A small molecule GPR40 antagonist, at a concentration of 10 μM, completely inhibited the stimulatory effect of 0.5 mM palmitic acid on glucose induced insulin secretion in C57/BL6J mouse islets.
Conclusion: Palmitic acid robustly potentiates glucose induced insulin secretion in mouse islets and this effect is inhibited by GPR40 knock-out or a GPR40 antagonist. These data indicate that acute stimulation of glucose induced insulin secretion by palmitic acid occurs exclusively via GPR40 signaling.

0503

A small molecule GPR40 antagonist inhibits fatty acid induced signaling and insulin secretion

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Background and Aims: GPR40 is a GPCR that is preferentially expressed in the pancreatic β-cells. Medium and long chain fatty acids have been shown to stimulate insulin secretion through the activation of GPR40. GPR40 null mice are protected against the detrimental effects of a high fat diet and have improved glucose tolerance compared with wild type mice fed a high fat diet; whilst overexpression of GPR40 in the β-cells of mice leads to impaired β-cell function, hypoinsulinaemia and diabetes. These data suggest that chronic fatty acid stimulation of insulin secretion is detrimental and that a GPR40 antagonist would be beneficial in the chronic treatment of type 2 diabetes. Here we present data detailing the in-vitro pharmacology of a novel, selective, small molecule GPR40 antagonist.
Materials and Methods: The HEK293 cell lines engineered to express the receptors (GPR40 or GPR10) were loaded with Calcium 3/Fluo 4 (Molecular Devices) in HBSS, 10 mM HEPES with 0.01%BSA. The cells are incubated for one hour and loaded onto the FLIPR (Fluometric Imaging Plate Reader). Compounds were added and assessed for their ability to inhibit the response to the EC80 concentrations for elaidic acid or PrRP respectively. To examine the effect of the antagonist in β-cells, insulin secretion was measured in MIN6 cells in the presence of 20 mM glucose. Following two hours incubation insulin was measured using an ELISA assay.
Results: The antagonist completely blocked the increase in intracellular calcium caused by stimulation with 22.5 μM elaidic acid, in HEK293s cells expressing GPR40, with a mean IC50 of 60 nM (n=30). It had no effect in HEK293s cells expressing another Gαq receptor GPR10 or in null cells. It completely inhibited elaidic acid stimulated GTPγS binding in GPR40 expressing membranes with a mean IC50 of 52 nM (n=11). In MIN6 cells, the antagonist completely inhibited the insulin secretion effects of a novel, small molecule GPR40 agonist and oleic acid.
Conclusion: Elaidic, oleic and the GPR40 agonist all robustly potentiated Ca++transients in the FLIPR assay and increase binding in the GTPγS assay. These compounds increased GSIS in MIN6 cells. These effects were inhibited by the small molecule GPR40 antagonist. This compound had no effect on PrRP responses in GPR10 expressing cells.
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0504

Glutamate is not co-secreted with insulin and glucagon in isolated rat beta and alpha cells

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Background and Aims: Glutamate release from rat islet α- and β-cells has been suggested to regulate glucagon and insulin secretion by autocrine and paracrine mechanisms. In particular, it has been reported that glutamate is co-secreted with glucagon from secretory granules. To study the role of glutamate in the endocrine pancreas, we measured insulin secretion in intact islets and purified β-cells as well as glucagon secretion from isolated α-cells and simultaneously monitored glutamate release.

Materials and Methods: Collagenase-isolated, free floating rat islets as well as FACS purified, attached rat α-and β-cells were cultured over night in RPMI-1640 containing 2 mM glutamine. All preparations were washed and incubated for 30 minutes in modified Krebs-Ringer-bicarbonate-Hepes buffer (0.05% BSA) at 2.5 mM glucose with various additions. Glutamate secretion was measured by a sensitive fluorescence enzyme cycling assay. Released glucagon and insulin were assessed by standard radio-immuno assays.

Results: In whole islets, 16.7 mM glucose stimulated insulin secretion 2.5 fold, while glutamate release was not significantly affected. However, glutamate content was increased by 38% ($p<0.01$). In isolated β-cells, insulin secretion was increased significantly by the following compounds: glucose (16.7 mM; 44%), leucine (10 mM; 105%), alpha-ketoisocarproic acid (KIC; 10 mM; 161%) and monomethyl succinate (mmsucc; 5 mM; 142%), which stimulate mitochondrial metabolism, as well as by the K_{ATP} channel-closing agent tolbutamide (100 uM; 47%). However, only glucose raised glutamate secretion (38%; $p<0.01$). Epinephrine (1 uM) abolished glucose-stimulated insulin and glutamate release but did not alter basal secretion rates. Consistent with our previous results, glucose, mmsucc and tolbutamide increased glucagon secretion from purified α-cells by respectively 28% ($p<0.001$), 37% and 44%, while leucine and KIC had no effect. Again in this cell type, glutamate secretion was enhanced only by glucose (47%; $p<0.001$). Remarkably, glutamate release was reduced by KIC (40%; $p<0.02$) and by epinephrine (32%; $p<0.05$). The latter agent stimulated glucagon release (50%; $p<0.01$). Inhibition of the mitochondrial dicarboxylate carrier by phenylsuccinate (10 mM) abolished glucose-induced glutamate release in β- but not in α-cells.

Conclusions: Glucose, but not other mitochondrial substrates, stimulates glutamate release, both in α-and β-cells. This involves mitochondrial transport in β-cells. In α-cells, glutamate production is markedly reduced by KIC, which drives the branched-chain amino acid transaminase reaction towards leucine, thereby resulting in glutamate depletion in these cells, in which glucose oxidation is less pronounced than in β-cells. It is unlikely that the reduction of glutamate release in both islet cell types by epinephrine results from inhibition of exocytosis, as even strong stimulation of hormone release is not associated with glutamate release. It remains to be established by which mechanism glutamate is released into the extra-cellular space from islet cells.

PS 28

Novel approaches to restore beta cell mass

0505

Intracellular signaling in Reg-induced pancreatic beta cell regeneration: involvement of the PI(3)K/ATF-2/cyclin D1 signaling pathway in the Reg/Reg receptor system

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Background and Aims: We have found an autocrine/paracrine beta-cell regeneration factor, *regenerating gene* protein (Reg), that increases the beta-cell replication. *Reg* is induced in pancreatic beta-cells by inflammatory stimulation such as by IL-6/glucocorticoids and acts as an autocrine/paracrine growth factor for beta-cell regeneration via a cell surface Reg receptor to ameliorate experimental diabetes. Recently, we produced *Reg* transgenic mice. The islets from the transgenic mice expressing Reg in beta-cells showed increased the [^{3}H]thymidine incorporation, and the development of diabetes in the non-obese diabetic (NOD) mice carrying the *Reg* transgene was significantly retarded. However, the manner by which Reg induces beta-cell regeneration was unknown.

Materials and Methods: RINm5F beta-cells were stimulated by Reg. Signal transduction pathways in pancreatic beta-cells by Reg stimulation were analyzed by using PathDetect *in vivo trans*-reporting systems. The levels of cyclin D1, phospho-ATF-2, and phospho-Rb were mea-

sured by Western blot analyses. Characterization of Reg-stimulated cyclin D1 promoter was carried out by reporter, EMSA, and ChIP assays. *Reg*$^{-/-}$ mice were produced by homologous recombination.

Results: ATF-2, but not CREB, cJun, Elk1, CHOP or cFos, was activated by Reg stimulation. The phospho-ATF-2 bound to −57~−52 of the cyclin D1 gene and the Reg-induced activation of the cyclin D1 promoter was inhibited by the cotransfection of the dominant-negative ATF-2 expression plasmid. The addition of PI(3)K inhibitors, LY294002 and wortmannin, attenuated the Reg/ATF-2-induced cyclin D1 promoter activity whereas SB203580, a p38 mitogen-activated protein (MAP) kinase inhibitor, PD98059, an inhibitor of the MAP kinase/ERK kinase-ERK pathway, KN-62 and KN-93, inhibitors of Ca^{2+}/calmodulin (CaM)-dependent protein kinase II, K-252a, an inhibitor of CaM kinase IV, and H-89, an A-kinase inhibitor, showed a negligible inhibitory effect on the cyclin D1 promoter activity in beta-cells. The immunoprecipitated PI(3)K phosphorylated ATF-2 in a time-dependent and dose-dependent fashion, indicating that PI(3)K directly phosphorylates ATF-2 to activate the cyclin D1 promoter. Furthermore, We produced *Reg*-knockout mice by homologous recombination. In *Reg*$^{-/-}$ mice, no Reg expression was detected in the pancreas and therefore no Reg was secreted into the islet culture medium. The BrdU incorporation in *Reg*$^{-/-}$ islets in the culture medium was significantly reduced compared to that in *Reg*$^{+/+}$ islets. We then analyzed the levels of phospho-ATF-2, cyclin D1 protein, and phospho-Rb in the islets. *Reg*$^{+/+}$ islets secreted Reg in the culture medium and the phospho-Rb level in the islets was much higher than that in *Reg*$^{-/-}$ islets. As expected, both the levels of phospho-ATF-2 and cyclin D1 were decreased in islets from *Reg*$^{-/-}$ mice, whereas the levels of other proteins such as PARP and CD38 as controls were unchanged.

Conclusion: These results indicate that the Reg–RegR system stimulates the PI(3)K/ATF-2/cyclin D1 signaling pathway to induce beta-cell regeneration.

0506

Role of protein kinase B isoforms in the regulation of functional β cell mass

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Background and Aims: Total β-cell mass can adapt according to metabolic demand. This important mechanism often prevents the onset of hyperglycaemia in the face of persistent insulin resistance, a condition leading otherwise to type 2 diabetes. The IRS2/PI3K/PKB signaling axis is considered to be required for this compensatory regulation by promoting survival, growth and proliferation of β-cells as well as inhibiting apoptosis. The aim of the present study was to elucidate the role of PKB isoforms in regulation of islet phenotype.

Material and Methods: Phenotypic analysis of mice (between one and three months old) deficient for either of the three PKB isoforms and comparison with heterozygous and wild type littermates: assessment of body weight and random blood glucose levels, immunostaining of pancreatic sections for insulin and glucagon to determine islet size and number, β-cell size and number, α-cell number.

Results: *Pkb*α$^{-/-}$ mice were smaller (males 22.6±0.6 g, n=6, *P<0.05*; females 19.1±1.3 g, n=3, n.s.) and displayed lower blood glucose levels (males 6.5±0.8 mM, *P<0.05*; females 5.8±0.1 mM, n.s.) compared to control littermates (males 26.4±1.2 g and 9.45±0.35 mM, respectively, n=5; females 21.5±0.8 g and 6.8±0.4 mM, n=5). *Pkb*β$^{-/-}$ mice had significantly higher blood glucose (males 9.3±1.0 mM, n=11, *P<0.05*; females 7.4±0.3 mM, n=6, *P<0.05*) compared to controls (males 7.4±0.6 mM, n=14; females 6.3±0.3 mM, n=7), whereas there was no difference in blood glucose levels between *Pkb*γ$^{-/-}$ mice and controls. Morphologic analysis of the pancreas revealed increased islet number (1.27 fold, n.s.) and size (1.91 fold, *P<0.05*) in *Pkb*β$^{-/-}$ females (n=3, controls n=4). A trend towards increased islet size (1.38 fold, n.s.) was also found in *Pkb*α$^{-/-}$ males (n=5, controls n=5), whereas no differences were detected for the other genotypes.

Conclusions: Unlike in IRS2-deficient mice, which show islet hypoplasia (and develop diabetes), no decrease (or on the contrary even an increase) in β-cell mass was observed in PKB-deficient mice. Our results suggest that all three PKB isoforms are individually dispensable for the maintenance of islet mass. This could be due to redundancy between isoforms or could depend on other signaling elements downstream of IRS2. While *Pkb*β$^{-/-}$ mice revealed hyperglycaemia and compensatory islet hyperplasia/hypertrophy, *Pkb*α$^{-/-}$ mice displayed increased islet mass in combination with decreased blood glucose levels which might indicate deregulation of islet growth without need for compensation (insulin resistance). This suggests that PKBα and β isoforms are individually not necessary in β-cells to increase islet mass.

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0507

A study of proliferation and prevention of cytokine-induced apoptosis in human beta cells

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Background and Aims: Type 1 diabetes mellitus (T1DM) is caused by a specific destruction of pancreatic beta cells. This pathogenic process is immune-mediated and it is well-known that the pro-inflammatory cytokines IL-1β, IFNγ and TNFα are important mediators of this beta cell death. An effective curative therapy for T1DM could be islet transplantation, although, shortage of islet donors poses a major problem to this therapy. Induction of human beta cell proliferation *in vitro* could solve this problem, however, human beta cells hold a very low potential for proliferation *in vitro* under conventional culturing methods. It has been established that GH, STAT proteins and GLP-1 are able to induce proliferation and protection against apoptosis of beta cells from rats and mice. Similarly, it is known that suppressors of cytokine signaling (SOCS) proteins can protect rodent beta cells from cytokine mediated apoptosis. The aim of this study is to induce proliferation of human beta cells and protect human beta cells from cytokine-induced cell death by exposing free-floating human islets or human islets cultured on extracellular matrix (ECM) to growth factors (including GLP-1 and GH) and/or by expression of SOCS-3 and STAT-5 proteins.
Materials and Methods: Isolated human islets with 40–90% purity were used. Because of limited supply of human islets, neonatal rat islets were used for optimisation. GFP-encoding Adenovirus was used to investigate and optimise transduction efficiency. Intact free-floating human islets or intact islets cultured on ECM (Biological Industries, Israel) were exposed to growth factors and/or transduced with STAT or SOCS-3 encoding Adenovira. Beta cells were identified by Pdx-1 and/or insulin staining and proliferation was analysed by BrdU incorporation. Apoptosis was induced by a combination of IL-1β, IFNγ and TNFα and was detected by TUNEL assay. Immunostaining was analysed by use of confocal or conventional microscopy.
Results: In intact rat islets GH induced a 10-fold increase in beta cell proliferation. Transduction of rat islets with constitutive active (CA) STAT-5 encoding Adenovirus induced a 3-fold increase in BrdU incorporation in beta cells. In free-floating intact human islets many BrdU positive cells were observed in islets transduced with CA-STAT-5 Adenovirus or in islets stimulated with GLP-1 and GH, both in the periphery and in the center of the islets. However, these cells did not co-express Pdx-1 and their identity is presently unknown. In human islets cultured on ECM, BrdU incorporation was observed in beta cells following stimulation with GLP-1 and GH. The possible role of growth factors, STAT-5 and SOCS-3 on protection from cytokine-induced apoptosis is currently being investigated.
Conclusion: Although human beta cells have a low potential for regeneration these studies indicate that proliferation can be induced by the combined use of ECM, stimulation with growth factors and/or expression of growth controlling signaling molecules including STAT-5.
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0508

IGF-1 protects insulin-secreting cells against glucocorticoid-mediated cell death via activation of MAP kinases but not PI3 kinase

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Background and Aims: Patients under long term treatment with immunosuppressive glucocorticoids may develop diabetes mellitus. Glucocorticoids are known to inhibit insulin secretion. Previously, we have shown that dexamethasone (dex), a synthetic glucocorticoid, induces apoptosis in β-cells. IGF-1, a potent stimulator of cell growth and an inhibitor of cell death, has been described to support insulin secretion and to induce β-cell proliferation which maintains β-cell mass. The present study examines whether IGF-1 protects insulin secreting INS-1 cells against dex-induced inhibition of proliferation and activation of cell death.
Materials and Methods: INS-1 cells were cultured under standard conditions in the presence of test substances for 1 to 4 days. Apoptosis was quantified by DAPI-stained condensed nuclei, by TUNEL assay and by caspase-3 activity. Proliferation was measured by BrdU-staining. The amount and phosphorylation of proteins (Bcl-2, PKB and IRS-2) was quantified by Western blotting. To differentiate distinct pathways activated by IGF-1 the inhibitors LY294002 of PI3-kinase, SB203580 of p38βMAP-kinase and PD98059 of p42/p44 MAP-kinase were used.
Results: Culture of INS-1 cells in the presence of dex (100 nM, 24 h) inhibited glucose (increased from 5 to

11 mM)- and IGF-1 (50 ng/ml)-induced cell proliferation. At the same time, dex reduced IGF-1 mediated phosphorylations of IRS-2 and PKB/Akt. Dex-stimulated cell death was reduced by IGF-1 by 75 and 58% after 1 and 4 days, respectively. While LY294002 abolished IGF-1 mediated phosphorylation of PKB/Akt and proliferation, it did not counteract but improve the protective effect of IGF-1 on cell death. The addition of MAP-kinase inhibitors, SB203580 and PD98059 antagonized the protection of apoptosis by IGF-1. Dex reduced the anti-apoptotic protein Bcl-2 an effect that persists in the presence of IGF-1.

Conclusion: These data suggest that IGF-1 stimulates proliferation of INS-1 cells via PI3-kinase pathway. The protective effect of IGF-1 against dex-induced apoptosis occurs in the absence of PKB/Akt phosphorylation and is mediated via MAP-kinase pathways.

0509

The anti-inflammatory cytokine interleukin-1 receptor antagonist protects from nutrition-induced hyperglycaemia and impaired glucose tolerance

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Background and Aims: In type 2 diabetes, chronic hyperglycemia has been associated with impaired beta-cell function and beta-cell apoptosis. This is partly mediated by beta-cell secretion and production of the pro-inflammatory cytokine IL-1beta. Previous studies indicate that the interleukin-1 receptor antagonist (IL-1Ra) protects human islets from the deleterious effects of high glucose and IL-1beta. Therefore, in the present study we investigated a possible protective role of IL-1Ra in hyperglycemic mice *in vivo*.

Materials and Methods: Six-week-old C57Bl/6 mice were fed a high fat/high sucrose diet (HFD) or a control diet for 12 weeks. Mice were daily injected with 10 mg/kg body weight IL-1Ra or solvent. Body weight and food consumption were measured weekly, intra-peritoneal glucose and insulin tolerance tests (ipGTT/ipITT) were performed every 4 weeks. After 12 weeks, mice were sacrificed and their islets isolated.

Results: As expected, the HFD led to a rapid weight gain compared to normal chow ($p<0.01$ after two weeks of diet). IL-1Ra injections did not significantly change body weights in either control or HFD mice. HFD feeding induced impaired glucose tolerance already after 4 weeks, which was inhibited in the IL-1Ra group after 4, 8 and 12 weeks of diet ($p<0.05$). After 8 and 12 weeks, the HFD fed control but not the IL-1Ra treated mice displayed hyperglycemia (blood glucose 11.75+/− 0.83 mM versus 7.52+/− 0.38 mM in control HFD versus IL-1Ra HFD mice after 12 weeks of diet, $p<0.001$). As predicted, serum insulin measurements revealed hyperinsulinemia induced by the HFD (0.77+/−0.23 ug/l versus 2.97+/− 0.46 ug/l insulin in control versus HFD mice). IL-1Ra increased basal as well as glucose stimulated insulin levels during an ipGTT in the normal diet group (4.11-fold increase in basal and 2.66-fold increase in stimulated serum insulin levels in normal diet mice, $p<0.01$). IPITT exhibited insulin resistance in the HFD group while IL-1Ra rendered them more insulin sensitive ($p<0.01$ after 12 weeks of diet). Glucose-stimulated insulin secretion (GSIS) assays of isolated islets showed that isolated islets from the HFD group lost glucose sensitivity compared to islets isolated from the normal diet group, which was improved by administering IL-1Ra (1.64+/− 0.58 stimulatory index in control HFD islets versus 2.16+/− 0.36 in the IL-1Ra HFD group).

Conclusion: These data show a protective role of IL-1Ra against nutrition-induced diabetes. This is in line with our previous study showing that IL-1Ra inhibits the deleterious effects of chronically elevated glucose or Interleukin-1β on isolated islets.Our results suggest a potential role for IL-1Ra in the treatment of diabetes.

0510

Augmented insulin secretion in the assessment of pancreatic β cell mass in healthy volunteers and patients with type 2 diabetes

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Background and Aims: Insulin secretion, a product of β-cell mass and function, decreases with the progression of

diabetes (DM), possibly due to decreasing β-cell mass. Methods used to investigate insulin secretion are often conducted on the background of changing insulin sensitivity, therefore failing to distinguish between improved function and increased mass. Previously, we developed a β-cell mass assessment procedure (β-MAP) in healthy volunteers (HV) that appears to maximally stimulate β-cell insulin secretion and is robust to changes in peripheral insulin sensitivity (Porksen *et al.* 2005 ADA Scientific Sessions, 1536-P). The aim of this study was to assess this novel β-MAP and its reproducibility in HV and patients with type 2 DM at various stages of disease severity.

Materials and Methods: Eight HV [median (range) age: 28(21–45 yr); BMI 24.8(19.7–27.5 kg/m^2)] and patients from each of 3 investigator-defined grades of type 2 DM severity [Grade 1: Dietary/exercise control (n=8), Grade 2: Treated with oral medication(s) (n=8), Grade 3: Treated with insulin (n=7); age: 54(37–65 yr); BMI: 25.4(18.6–29.9 kg/m^2)] participated in this open-label, 2-period, replicate study. The β-MAP consisted of the following sequence of manoeuvres; a loading glucose bolus (25 g of 50% dextrose); 45 min fixed-rate glucose infusion (600 mL/h of 20% dextrose) to induce hyperglycaemia (approx. 22 mmol/L); and an i.v. glucagon bolus (1 mg) administered 15 min after the start of glucose infusion. Glucose delivery was titrated lower if blood glucose levels exceeded 30 mmol/L. Blood samples were collected for glucose, insulin, and C-peptide.

Results: Mean glucose levels at baseline and after administering glucagon, and mean baseline insulin levels were significantly higher for all DM groups compared to the healthy subjects. Prior to the glucagon bolus, the hyperglycaemic stimulus failed to increase insulin secretion in diabetics. The maximal insulin level and overall insulin exposure over 30 min after the glucagon bolus [insulin C_{max}(15–45 min) and AUC(15–45 min)] were significantly lower for the 3 DM grades when compared to the HV. Insulin C_{max}(15–45 min) and AUC(15–45 min) trended lower with increasing severity of the DM, with C-peptide levels demonstrating a similar trend. The intra-subject CV% for insulin C_{max}(15–45 min) and AUC(15–45 min) in all groups ranged from 8 to 33.3%. Insulin C_{max}(15–45 min) correlated well with HOMA2%B (β-cell function index) (R^2=0.514, p<0.001) but not HOMA IR (insulin resistance) (R^2=0.010).

Conclusion: The novel β-MAP demonstrated a clear separation of insulin response between HV and DM patients, and appears well correlated with an estimate of β-cell mass (HOMA2%B) but not with insulin resistance, as indirectly estimated by HOMA IR. The low intra-subject variability suggests that the procedure is reproducible and may be a feasible tool in a clinical setting to monitor changes in β-cell mass.

Parameter Group	Control Group (Least-squares Geometric Mean)	DM Group (Least-squares Geometric Mean)		Ratio to Control (90% confidence interval)	P-value for Difference to Control
Insulin C_{max} (15–45 min) (pmol/L)	4385	DM Grade 1	1418	0.32 (0.20, 0.52)	0.001
		DM Grade 2	781	0.18 (0.09, 0.34)	0.001
		DM Grade 3	593	0.14 (0.07, 0.26)	0.001
Insulin AUC (15–45 min) (pmol*h/L)	1582	DM Grade 1	393	0.25 (0.15, 0.41)	<0.001
		DM Grade 2	208	0.13 (0.07, 0.25)	<0.001
		DM Grade 3	184	0.12 (0.06, 0.21)	<0.001
C-peptide AUC (15–45 min) (pmol*h/L)	2927	DM Grade 1	1278	0.44 (0.33, 0.59)	<0.001
		DM Grade 2	986	0.34 (0.22, 0.51)	0.001
		DM Grade 3	921	0.31 (0.22, 0.44)	<0.001

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0511

Monoclonal autoantibodies in diabetes and their potential use in islet beta-cell Imaging: the BB-rat monoclonal IC2 further purified and fragmented for improved performance in non-invasive *in vivo* imaging

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Background and Aims: Since early 1980ths several monoclonal autoantibodies have been discovered from type-1 diabetic experimental animals (C57BLKsJ db/db-mice, NOD-mice, BB-rat) and from human diabetic patients. Among these autoantibodies are mainly insulin (K14D10), islet cell, neural and lymphoid cell autoantibodies described. Several hybridomas with specificity against gangliosides (A2B5, R2D6), sulfatides (IC2) and other cellular targets (3A4, 5C12, GAD and IA-2) have been described. Both target autoantigens and the immunogenic epitopes has been fully explored. In addition recombinant anti-GAD and anti-IA-2 have been made.

Serum autoantibodies against these epitopes are seen across species, and increased titres of these specificities are used as prediagnostic tool for type-1 diabetes, however, without being strictly specific for type-1 diabetes. Attempts have been done within the last 6 years to use some of these monoclonal autoantibody specificities for pancreatic beta-cell imaging. The K14D10 anti-insulin autoantibody failed to behave specific to the beta-cell in scintigraphy by gamma-imaging, but the IC2 monoclonal BB-rat autoantibody performed highly promising for a non-invasive *in vivo* imaging of the beta-cell mass with its pronounced specificity to the beta-cell surface. Being a strict beta-cell surface specific rat IgM-kappa autoantibody with a target molecule (sulfatide) known to be present also on islet cells innervating Schwann cells and other neural tissues, we still need fully to explain the unique beta-cell surface specificity of IC2.

Materials and Methods: To further explore the molecular specificity of IC2 we need large amount of purified IC2 IgM autoantibody for fluorochrome labelling, fragmentation, and radioisotope labelling. Affinity purified IC2 was obtained from several litres of IC2 culture supernatant using a column of MARK-1 (mouse anti-rat kappa) conjugated to Sepharose. The eluted rat IgM-kappa was further fragmented to create IC2 F(ab)2 using standard protocols with pepsin digestion. Finally, the digest was affinity purified on Protein L-Sepharose to remove the Fc fragment. The entire IC2 IgM-kappa, and the F(ab) 2-kappa is radioisotope labelled by the DTPA-method, and the labelling efficiency of the antibody-amide-DTPA-indium-111 conjugate was studied by autoradiography. In vivo non-invasive imaging experiments come next.

Results: The Integra bioreactor CELLine CL 1000 is used for large scale production of IC2 containing culture supernatant. After appropriate purification by MARK-1 (mouse anti-rat kappa) affinity chromatography, the purified IC2 is fragmented by pepsin digestion and the Fc and F(ab) 2 fragments further separated by Protein L-Sepharose affinity chromatography. These purified entire IgM and F (ab)2 is currently labelled with fluorochrome for immunofluorescence studies and Indium-111 for medical non-invasive imaging by gamma camera.

Conclusion: The monoclonal autoantibody IC2 is now fully characterized and made commercial available though contact with the presenting author. According to previous and recent studies the Indium-111-labelled IC2 is so far the only monoclonal autoantibody available for non-invasive beta-cell mass imaging. Its eventual reactivity to islet Schwann cells is currently under investigation by immunofluorescence microscopy.

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0512

Effects of hyperglycaemia on human pancreatic islet endothelial cells

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Background and Aims: High glucose induces pathological alterations in small and large vessels, responsible for micro and macrovascular complications in both type 1 and 2 diabetes and attributed to endothelial cell damage. The underlying mechanisms have not been fully elucidated and data are mainly derived from studies on HUVEC. In this study we aimed to analyse the effects of sustained hyperglycemia on human pancreatic islets microendothelial cells (MECs), themselves exposed to high glucose concentrations in the course of the disease. Islet MECs have been shown to have peculiar characteristics, including the expression of the transmembrane barrier protein nephrin, which has signaling functions, and possibly they are involved in fine-tuning islet blood glucose sensing and regulation. We analysed the effects on cell survival and proliferation, cell cycle, and apoptosis, with particular regard to the PI3K/Akt pathway.

Materials and Methods: Human pancreatic islet MECs were extracted and purified using anti-CD105 coated immunomagnetic beads, characterised and cultured in normal (5.6 mmol/L) or high (28 mmol/L) glucose in appropriate medium for up to approximately 30 days. Cell proliferation was assessed by cell count and DNA synthesis by BrdU incorporation assay. Apoptosis was evaluated at 3–6 days intervals, by enzyme immunoassay measuring mono- and oligo-nucleosomes in the cytoplasmic fraction of cell lysates, index of DNA fragmentation, and by detection of Caspase-3 activity. Moreover, by WB analysis, the antiapoptotic Akt activity and the P-Akt/Akt ratio, as well as the effects on expression and phosphorilation of nephrin were evaluated.

Results: Islet MECs cultured in high glucose retained endothelial characteristics, assessed by flow cytometric analysis but were significantly less numerous than those in physiological glucose concentration (proliferation was reduced of 12.7% after 2 days, 19% after 7 days, 23.8% after 12 days, 47.7% after 25 days, when compared to cells in normal glucose). In parallel experiments apoptosis progressively increased, from 7% after 2 days to 79% after 30 days in high glucose, compared to normal glucose culture. This was accompanied by increased caspase-3 activity, up to 300%, mainly during the first days of culture. Moreover, high glucose culture persistently reduced Akt

activity and P-Akt/Akt ratio, accompanied by inhibition of nephrin tyrosine phosphorylation.

Conclusion: Hyperglycemia of several days duration induces apoptosis in islet MECs. Due to their emerging role in islet development and function, these effects might be relevant to beta cell mass and responsiveness and in the pathophysiological processes of type 1 and type 2 diabetes. The underlying mechanisms involve a reduction of the serine-threonine kinase Akt, which serves as a multifactorial cell regulator and that, in beta cells, has been shown to result in impaired glucose tolerance due to defective insulin secretory pathway. Impaired nephrin phosphorylation, that is a critical step in nephrin induced signaling and in stimulation of Akt-dependent pathways also appears to be relevant. Our findings suggest these mechanisms as target of potential intervention strategies in improvement of beta cell function.

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PS 29

Novel signaling molecules in beta cell function and dysfunction

0513

Analysis of the beta cell and insulin-containing vesicle proteome

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Background and Aims: Beta-cells of the pancreatic islets of Langerhans act as glucose sensors, adjusting insulin secretion to the prevailing blood glucose concentration. Triggering of secretion involves increases in intracellular ATP concentrations and Ca^{2+} influx through voltage-gated Ca^{2+} channels which underlie the acute, or first-phase of insulin release. In contrast, the molecular basis of K_{ATP}-channel independent secretion is poorly understood while large dense core vesicle (LDCV) recruitment is thought to be a vital component of this process. Both microtubule and actin-dependent mobilisation of vesicles are required for sustained release of insulin through mechanisms which have yet to be fully elucidated. The aim of this study was to carry out systematic analysis of the insulin-granule proteome – which information is essential to determine the signaling pathways involved in glucose-induced LDCV recruitment and secretion.

Materials and Methods: MIN6 pancreatic beta-cells were homogenised and subcellular fractionation was performed using Optiprep™ gradients. Fractions were analysed for by immunoblotting using specific antibodies for organelle markers and by measuring their insulin content. Two complementary 2-dimensional gel electrophoresis methods were used: isoelectric focusing (IEF)/SDS-PAGE and benzyl hexadecylammonium chloride (16-BAC)/SDS-PAGE to obtain the best coverage of the LDCV proteome. Proteins were excised from the gels, digested and peptides identified by MALDI-TOF mass spectrometry using the MASCOT database search.

Results: The density gradient centrifugation resulted in highly enriched LDCV fractions. The IEF/SDS-PAGE method provided superior resolution of soluble proteins while the 16-BAC/SDS-PAGE improved separation and resolution of integral membrane proteins. So far 24 proteins were identified including the heat shock cognate protein (HSc70) – involved in un-coating of clathrin-coated vesicles; vacuolar ATP synthase (v-ATPase) subunits, which maintain a low intravesicular pH, important for proinsulin conversion and storage of zinc-insulin hexamers; valosin-containing protein – an NSF homologue involved in exocytosis; glucose regulated protein 54, 78 and 94. Several proteins not directly attached to vesicles but involved in vesicle cycle regulation copurified with vesicles for example actin and tubulin. Both actin and tubulin form core complex with synapsin proteins during vesicle docking thus explaining their presence on insulin vesicles.

Conclusion: Systematic proteomics analysis has revealed the molecular composition of insulin granules. Alteration in the composition and phosporylation status of LDCV proteome induced by glucose will provide fundamental information on the molecular basis and regulation of insulin-granule function. Better understanding of the downstream targets of glucose-derived metabolic signals on LDCVs likely to contribute to the development of a novel, more targeted therapeutic approach for type 2 diabetes.

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0514

CART, a new player in the regulation of islet hormone secretion

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Background and Aims: CART is an anorexigenic peptide widely expressed in the central and peripheral nervous systems as well as in islet endocrine cells, including beta cells during rat development and delta cells of adult rats. CART null mutant mice display impaired glucose-stimulated insulin secretion *in vivo* and *in vitro*, together with impaired glucose elimination and reduced expression of GLUT-2 and PDX-1. In addition, a mutation in the human CART gene cosegregates with obesity and type 2 diabetes.
Materials and Methods: We examined the effect of CART 55–102 on islet hormone secretion, using INS-1 (832/13) cells and isolated rat islets. In addition, islet CART expression was examined in two rat models of type 2 diabetes: Goto–Kakizaki (GK) rats and dexamethasone treated rats.
Results: At high glucose CART potentiated cAMP stimulated insulin secretion via a further increase in cAMP and a subsequent stronger activation of the protein kinase A dependent pathway. In the absence of cAMP-elevating agents, CART was without effect on INS-1 cells but inhibited secretion of insulin, glucagon and somatostatin from isolated islets. CART was markedly upregulated in the beta cells of both diabetes models. Thus, in dexamethasone treated rats beta cells expressing CART mRNA and peptide were 10-fold increased compared with control rats. In GK rats the relative number of CART expressing beta cells was 30-fold higher than in Wistar control rats. Electron microscopy localised CART to the secretory granules of the beta cells, indicating that CART is co-secreted with insulin. In addition, GK-rats displayed a greater secretory response to CART than Wistar controls. Western blot analysis of CART in normal and diabetic islets revealed that islet CART had molecular weight similar to CART 55–102.
Conclusion: We conclude that 1) CART is a regulator of islet hormone secretion, acting through a receptor coupled to adenylate cyclase. 2) CART is upregulated in the beta cells of type 2 diabetic rats and is located to the secretory granules. 3) CART in normal and type 2 diabetic islets is biologically active. CART may therefore play important roles in glucose homeostasis and in the pathophysiology of type 2 diabetes.
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0515

E-cadherin-mediated cell adhesion: a role in islet architecture, cell-to-cell coupling and insulin secretion?

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Background and Aims: The current drive towards islet transplantation as a viable cure for type 1 diabetes has focused attention on the characterization and development of substitute tissue that responds appropriately to elevations in glucose. It is widely accepted that the pancreatic islet and not individual beta-cells represent the unit of insulin secretion, yet no clear evidence exists to explain why homotypic populations of cells within islets respond more effectively than individual beta-cells. Furthermore, heterogeneous individual responses to nutrients are usurped by concerted, synchronised glucose-responsiveness when cells are configured as an islet. Close association between cells in the islet is facilitated by the expression of cell adhesion molecules, including E-cadherin (ECAD) and beta-catenin which binds the cytoplasmic domain of the cadherin. These molecules not only hold the 3-dimensional islet together, but may offer an explanation for improved glucose-responsiveness. In the current study we have examined the importance of ECAD on synchronicity of activity and insulin secretion within model islets.
Materials and Methods: MIN6 were cultured either as monolayer (M) or pseudoislets (PI). Nutrient and non-nutrient-evoked changes in cytosolic calcium were determined from fura-2-loaded Ms and/or PIs cultured either in the presence or absence of the immuno-netralising ECAD-antibody or IgG control. Insulin secretion was measured via ELISA. Co-localization of beta-catenin and insulin was visualized using confocal microscopy, using Alexa-594/488 secondary antibodies. ECAD was over-expressed using Lipofectamine transfection with pcDNA3.1(-) vector containing the coding region of mouse ECAD gene. Expression levels were confirmed using Western blot analysis.
Results: MIN6 cells configured as PIs exhibit greater glucose (20 mM)-evoked increases in insulin secretion (802±73% over basal; n=7) compared to those cultured as monolayers to 80% confluence (147±14% over basal; n=6). Western blot analysis suggests that MIN6 cells within a PI express more ECAD than cells within a monolayer. Fura-2 microfluorimetry data confirms synchronicity of glucose-evoked calcium oscillations in PIs compared to the heterogeneous response of individual MIN6-cells (data from 6 separate experiments). Both islet morphology and synchronicity of nutrient-evoked calcium oscillations are altered in the presence of immuno-neutralising anti-ECAD, which prevents homophilic ECAD ligation. Beta-catenin and insulin immunoreactivity were co-localised only at the cell-to-cell interface in MIN6 monolayers. Up-regulation of ECAD expression in our MIN6 cells was confirmed following transfection using Western blot and confocal microscopy (20-fold enhancement; n=5). However, insulin secretion studies suggest that ECAD over-expression does not enhance glucose-stimulated insulin secretion from

MIN6 cells (over-expressing cells: 171±20% basal; n=3; vector control cells: 175±17% basal; n=3).
Conclusion: Our data suggest that ECAD is important in the formation and maintenance of islet architecture and that loss of ECAD uncouples glucose-evoked signaling within the islet. Over-expression of ECAD fails to enhance insulin secretion, suggesting that ECAD may act to mediate appropriate nutrient-evoked signaling, rather than control insulin secretion per se.
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0516

Mutant huntington impairs metabolism and GSIS in the 832/13 INS-1 cell line

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Background and Aims: Huntington's disease (HD) is a neurodegenerative disorder caused by an expansion of a CAG triplet repeat in the gene coding for the protein huntingtin. The disease develops when the resulting stretch of glutamine residues exceeds 36. One of the main pathological findings is formation of intracellular inclusions consisting of huntingtin and other proteins. In addition to neurological deficits, a relatively large proportion of HD patients suffer from diabetes, a condition that also develops in the most widely used animal model of the disease, the R6/2 mouse. We have previously found that R6/2 beta-cells exhibit intracellular inclusions, presumably causing impaired beta-cell replication and perturbed exocytosis. We now aim to further characterize the role of normal and mutant huntingtin in beta-cell function, using the highly glucose-responsive 832/13 cell line.
Materials and Methods: We overexpressed huntingtin with and without disease causing PolyQ lengths (17 and 69 Q, respectively) in 832/13 INS-1 cells and analysed the cells with regard to inclusion formation (immunocytochemistry), general metabolic activity (methyl-tetrazolium assay; MTS) and insulin secretion. In addition to the vectors mentioned above a control vector containing the gene for EGFP was also included in the study.
Results: Fluorescence microscopy showed that expression of 69Q huntingtin, but not 17Q huntingtin or EGFP, led to formation of intracellular inclusions 48 h after transduction in the 832/13 INS-1 cell line; inclusions were found both in the cytoplasm and in the nucleus, the latter form which is typically found in HD. This was accompanied by an overall reduction of metabolic activity, as measured by MTS-assay; 69Q huntingtin caused a 67% reduction relative to uninfected cells. This decrease is significantly larger than the one caused by expression of EGFP alone or 17Q huntingtin (47 and 45% respectively, $p<0.01$). In addition to this, glucose stimulated insulin secretion (GSIS) was also markedly reduced in response to expression of mutant huntingtin. When stimulating the 69Q expressing cells with 16.7 mM glucose, they displayed a dramatically reduced insulin secretion compared to cells expressing 17Q huntingtin (40.2 and 167.6 ng/h/mg protein, respectively; $p<0.001$). The fold change relative to secretion at 2.8 mM glucose was also reduced from 10.2 to 1.3.
Conclusion: The results show that overexpression of huntingtin containing an HD-causing PolyQ stretch in beta-cells leads to formation of intracellular inclusions. Concomitant to this there were marked disruptions of cellular function, as both metabolism and insulin secretion were dramatically perturbed.
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0517

Molecular mechanism of *db* gene induced pancreatic β cell dysfunction: Evidence for compensatory mechanism to reduce susceptability to diabetes in *db* gene heterozygote

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Background and Aims: The *db* gene is a splicing variant caused by a point mutation of the leptin receptor gene. The *db* gene homozygous BKS.Cg-+$Lepr^{db}$/+$Lepr^{db}$/Jcl(*db/db*) mice develop diabetes with a marked obesity, severe insulin resistance, and limited capacity of insulin secretion. The diabetes phenotype is not demonstrated in heterozygous mice (*db/+*). To clarify a molecular mechanism of pancreatic βcell dysfunction related with the *db* gene, the cell function and gene expression profile of pancreatic islets were analyzed in *db* gene homozygous *db/db*, heterozygous *db/+*, and lean littermates (+/+). Laser Capture Microdissection (LCM) and real-time RT-PCR were applied to analyze islet cell specific gene expression.
Materials and Methods: The male *db/db*, *db/+* and+/+were provided free access to standard feed. Body weight (BW), fasted blood glucose (FBG) and insulin (FIRI) were measured at the age of 8 and 12 weeks. To analyze gene expression profiles in β cells, the islet core region was selectively obtained by LCM. Primer pairs encoding genes associated with pancreatic hormones, cell proliferation, cell differentiation, apoptosis, cell cycle, ER/oxidative stress, and organization construction were prepared, and real-time RT-PCR with Sybr Green was applied. Each gene expres-

sion was relatively quantified by the comparative Ct method with each result in the+/+mice as a control.
Results: BW, FBG and FIRI in 8 and 12 week-old *db/db* mice were increased significantly compared with those in other groups. At 12 weeks of age, however, insulin content in the islet was markedly reduced in *db/db* mice and increased significantly in *db/*+mice comapered with+/+mice (5.1±3.3, 58.0±31.7, 24.4±15.4 ng/islet, respectively). A prominent increase of insulin 2 gene expression was demonstrated in *db/*+mice in comparison with *db/db* mice at 8 weeks of age (*db/*+: 9.57±3.96, *db/db*: 1.72±0.96, $p<0.05$), but any difference was not observed between both groups at 12 weeks of age (*db/*+: 7.86±3.56, *db/db*: 6.97±3.78). Expression of genes involved in cell proliferation (cyclin E & ERK1) and apoptosis (CAD) was accelerated in 8 week-old *db/db* mice. In 12 week-old *db/db* mice, cyclin E & ERK1 gene expressions were down-regulated and CAD gene expression was up-regulated. On the other hand, the bcl-2 gene expression was increased significantly in *db/*+mice. Expression of genes involved in anti-oxidation such as SOD2, catalase, and GSHPx were significantly decreased in *db/db* mice. To examine a role of *db* gene on glucose tolerance, *db/*+and+/+mice were fed high-fat-diet for 6 weeks from 10 weeks of age. A significant increase of BW was observed in mice fed high-fat diet compared with normal chow. The high fat diet did not impair glucose tolerance analyzed by ipGTT, and a significantly higher plasma insulin in response to glucose challenge was observed in *db/*+mice fed high fat diet, but not in+/+mice.
Conclusion: The islet gene expression profiles demonstrated that the *db* gene heterozygous mice have a protective mechanism against acceleration of apoptosis and reduction of cell proliferation observed in *db* gene homozygous mice. In addition, increase of islet insulin content and high response of insulin secretion to glucose load observed in *db/*+mice with high fat diet strongly suggest an existence of the compensatory mechanism in the *db* gene heterozygote to reduce a susceptibility to diabetes.

0518

Expression and function of GPR40 in Goto–Kakizaki rat islets

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Background and Aims: The recently characterized receptor GPR40 is abundantly expressed on pancreatic β-cells and is activated by medium to long chain fatty acids (FA). Receptor activation augments glucose-stimulated insulin secretion (GSIS) via the generation of the intracellular second messenger phosphatidyl inositol-3 phosphate (IP_3) and ensuing calcium release from the endoplasmatic reticulum. The Goto–Kakizaki (GK) rat is a commonly used model of spontaneous type 2 diabetes, with a number of established and proposed defects, created by repetitive inbreeding of hyperglycemic Wistar rats. The aim of this study was to investigate the expression and function of GPR40 in the islets of GK rats.
Materials and Methods: We used male Wistar and GK rats purchased from Taconic (Skensved, Denmark) and fed a normal diet. Pancreatic islets were isolated from the rats by collagense digestion. For mRNA expression analysis, islets from different individuals were kept separated and stored in −80°C pending analysis. RNA was prepared and 2 μg were used for cDNA synthesis and controls, after DNase treatment. Using real-time PCR, the expression of GPR40 mRNA between the different individuals was compared, after normalization against the islet mRNA expressions of three different house-keeping genes. In the phosphatidyl inositol (PI) hydrolysis experiments, islets from 2–4 animals were pooled and the divided into batches of 50, radioactively labelled with ^{3}H-inositol, and incubated for 30 min with or without 100 μM linoleic acid (LA). The islets were then lysed and the inositol phosphates (IPs) were collected using anion exchange chromatography. RIA analysis of FA augmentation of GSIS was performed on pooled islets in batches of 3 after 60 min of incubation at 3.3 mM glucose (0.1% BSA) in the presence or absence of a free LA concentration of 100 μM;.
Results: On the mRNA level, the expression of GPR40 in the GK rat islets was markedly downregulated compared with that of the Wistar controls (GK GPR40=40% of control, $p=0.0001$). These results were corroborated in the functional analyses. In the IP production assay, LA stimulation of Wistar rats resulted in an 85% increase of IP levels compared to unstimulated islets ($p=0.014$), while no significant effect of the fatty acid challenge was detected in the GK model. The FA-induced augmentation of GSIS from GK islets was also significantly lower than that in the controls animals. The addition of LA caused a 240% increase in secreted insulin ($p=0.013$) from the Wistar islets, whereas the effect of LA on the GK islets was not statistically significant.
Conclusion: Our results show that the previously reported expression of the fatty acid receptor GPR40 in Wistar rats is significantly attenuated in the GK rat. This deficiency makes GK islets less responsive to *in vitro* FA stimulation, as exhibited both by a perturbed hydrolysis of radioactive PIs and by an abolished effect on GSIS. The conclusion is that a reduction of GPR40 expression and function can be added to the list of mutations in the GK rat model of type 2 diabetes.
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0519

Identification of BACE (β-site amyloid precursor protein cleaving enzyme) expression and activity in pancreatic cells

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Background and Aims: BACE (β-site amyloid precursor protein cleaving enzyme) is a membrane-bound aspartyl protease that can process amyloid precursor protein (APP) at the β-secretase site, being responsible for amyloid β protein (Aβ) generation in brain. Although BACE mRNA expression is also found in pancreas, pancreatic BACE substrates are unidentified, and thus its pancreatic physiological role remains unknown. The aim of the study was to characterize expression and activity of BACE in pancreas, and testing influence of glucose estate.
Materials and Methods: Immunofluorescences against BACE were performed in paraffined pancreas sections. Cell lysates of 50 μg protein from human pancreatic islets and pancreatic β cell line MIN6 were immunoblotted for BACE expression, and tested for fluorometric BACE proteolytic activity assay with 20 μM fluorometric BACE substrate (JMV2236). Human pancreatic islets and MIN6 cells were evaluated after 5 days culture at either 5.5 or 22.7 mM glucose. Human pancreatic islets and MIN6 cells cultures were treated with 10 μM cell permeable BACE inhibitor (Z-VLL-CHO) for 24 h. Quantification of insulin transcript was performed by real time QRT-PCR using Taqman probe. Insulin peptide content was evaluated by immunoblot and quantified by ELISA.
Results: Islet cells showed higher expression levels of BACE compared to exocrine tissue in immunofluorescences of paraffined pancreatic sections. Protein extracts from human pancreatic islets exhibited BACE activity when these were tested in fluorometric BACE proteolytic activity assay (n=5, K_{obs}=0,166±0,02 sec^{-1}). Human pancreatic islets expressed three major bands of approx 55, 70 and 140 KDa in BACE immunoblots, identified as immature monomer, mature monomer and mature dimmer, respectively (n=6). Moreover, BACE was also expressed and catalytically active in protein extracts from MIN6 cells (n=8, K_{obs}=0,029±0,001 sec^{-1}). We then assessed whether BACE expression and activity could be regulated by glucose. Either human pancreatic islets or MIN6 cells cultured for 5 days at 22.7 mM glucose showed a 4-fold increase in the expression level of 70 KDa BACE compared to those at 5.5 mM (n=10, $p<0.001$). Instead of being *de novo* peptide maturation, the increase was originated by dissociation of the mature peptide dimmer. When BACE proteolytic activity was tested, a significantly reduction was observed in protein extracts from cultures at 22.7 mM compared to those at 5.5 mM glucose with K_{obs}=0,024±0,0004 sec^{-1} and K_{obs}=0,028± 0,001 sec^{-1}, respectively (n=4, $p<0.01$). To investigate the effects of reduced BACE proteolytic activity, Z-VLL-CHO was administrated. A 1.3 to 3.8 fold-decrease in insulin transcript expression were identified in MIN6 cells from 2 to 24 h (n=8, $p<0.001$), followed by a 1.4 to 2.2 fold-reduction in insulin content from 4 to 24 h of treatment compared to control (n=4, $p<0.05$). Moreover, treated human pancreatic islets showed a 4 fold-change reduction in the expression level of insulin peptide compared to control (n=3, $p<0.05$).
Conclusion: BACE activity and expression in pancreatic islet cells is regulated by the glucose state. Reduced BACE activity downregulates both insulin transcript expression and peptide content. Further identification of pancreatic BACE substrates would be needed to interpret the present results.

0520

Islet endothelial cell signaling augment beta cell function

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Background and Aims: The proximity of islet endothelial cells and beta-cells in the pancreatic islets by necessity means that they are exposed to each others products. Islet endothelial cells require signals from the beta-cells to function properly, but little is known about how paracrine signals from the endothelial cells affect the beta-cells. The present study tested the hypothesis that islet endothelial cell-derived products may affect adult beta-cell function.
Materials and Methods: Pancreatic islets from adult inbred Wistar-Furth rats were isolated. Islet endothelial cells from some of the islets were stimulated to proliferate and grow out on a collagen matrix, and were then purified with Dynabeads coated with the endothelial cell specific marker Bandeiraea simplicifolia, achieving a purity of the islet endothelial cells exceeding 90%. Islet conditioned medium was obtained by incubating other islets in RPMI 1640 with 10% fetal calf serum for 24 hours. Endothelial cell conditioned medium was obtained by incubating islet endothelial cells in RPMI 1640 with 10% fetal calf serum for 12 hours. Insulin release, insulin content, glucose oxidation and insulin biosynthesis was measured in batch-type experiments following exposure

of cultured islets incubated in these mediums, or control medium, for 24 hours.
Results: The islets incubated in endothelial cell conditioned medium released 50% more insulin than islets incubated in RPMI with 10% fetal calf serum alone when exposed to 16.7 mmol/l glucose (p<0,001; ANOVA). Moreover, the insulin content of islets exposed to endothelium conditioned medium was increased with 75% when compared to the same control group (p<0,01; ANOVA). However, the glucose oxidation rates and (pro)insulin biosynthesis in response to 16.7 mmol/l glucose did not differ between the groups. Further studies are now undertaken to determine the endothelial-derived factor responsible for the beneficial effects on beta-cell function, and its exact mechanism.
Conclusions: The islet vascular system seems to have other effects besides its transport capacities to augment and sustain beta-cell insulin content and secretion.
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PS 30

Lipid metabolism

0521

Acyl CoA: diacylglycerol acyltransferase 2 (DGAT2)-mediated triacylglycerol accumulation in glycolytic muscle promotes insulin resistance

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Background and Aims: Insulin resistance and type 2 diabetes are frequently accompanied by lipid accumulation in skeletal muscle. However, it is not known whether triacylglycerol (TG) deposition in skeletal muscle causes insulin resistance or whether the type of muscle fiber_-oxidative, red fibers or glycolytic, white fibers_is an important determinant of lipid-mediated insulin resistance. Oxidative muscle normally stores more TG and is more insulin-sensitive than glycolytic muscle, suggesting that increased TG content in oxidative muscle may not be detrimental. We hypothesized that TG accumulation in glycolytic muscle, which does not normally store large quantities of TG, would promote insulin resistance.
Materials and Methods: We generated transgenic mice that overexpress DGAT2, an enzyme that catalyzes triglyceride biosynthesis, in glycolytic muscle, by using the muscle creatine kinase (MCK) promoter.
Results: At 3 -months of age, MCK-*DGAT2* mice had a fivefold increase DGAT2 mRNA expression and a twofold increase in TG accumulation in glycolytic muscle. Although fasting plasma levels of glucose and insulin were similar in MCK-*DGAT2* and wild-type mice, MCK-*DGAT2* mice exhibited impaired glucose tolerance. The insulin-stimulated activity of PKCλ, a sensitive marker of insulin signaling that is highly correlated with glucose uptake in muscle, was severely reduced in glycolytic (but not oxidative) muscle in MCK-*DGAT2* mice. Insulin-stimulated uptake of 2-deoxyglucose was also severely impaired in glycolytic (but not oxidative) muscle. The ceramide content was increased, and diacylglycerol content was reduced specifically in the glycolytic muscle of MCK-*DGAT2* mice.
Conclusion: Our results indicate that the accumulation of moderate amounts of TG in glycolytic muscle impairs insulin sensitivity and glucose uptake and suggest that lipid deposition in nonoxidative, glycolytic muscle may contribute to insulin resistance and diabetes.
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0522

Heredity-independent effects of increased aerobic fitness on liver and adipose tissue FFA uptake

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Background and Aims: Regular physical exercise decreases body adiposity and improves whole body lipid and glucose utilization although the effects of increased physical activity on liver and adipose tissue free fatty acid (FFA) uptake are largely unknown. In the present study we studied heredity-independent effects of moderately increased physical activity and aerobic fitness on liver and adipose tissue FFA uptake in monozygotic twins and compared FFA uptake rates in different tissues *in vivo*.
Materials and Methods: Nine young healthy adult male monozygotic twin pairs with 18% (50.1±5.1 vs. 43.4±6.7 ml/kg/min, p<0.001) mean difference in maximal oxygen uptake

(VO_2max) were recruited from a large twin cohort study. Abdominal subcutaneous and visceral fat, hepatic, and muscle FFA uptake were measured at fasting state with positron emission tomography and [^{18}F]FTHA and fat masses were quantified using MRI. The co-twins with higher VO2max constituting the more active group were compared to the less active brothers with lower VO_2max.

Results: Both groups had similar BMI and similar lean body mass, but more active twins had 10% lower whole-body fat percent (17.6±5.5 vs. 19.6±5.7%, p<0.05). More active twins had 19% lower abdominal subcutaneous (1.88±0.97 vs. 2.37±1.06 kg, p=0.02) and 20% lower visceral fat mass (0.81±0.44 vs. 0.99±0.41 kg, p=0.009) than less active twins. Fasting plasma glucose and serum FFA concentrations were similar between the groups. When expressed per kilogram of tissue, hepatic FFA uptake was highest (7.23±5.43 μmol/100 ml/min), visceral adipose tissue FFA uptake was 30% higher than subcutaneous uptake (0.23±0.17 vs. 0.19±0.15 μmol/100 g/min, p=0.006) and correlated with subcutaneous, hepatic and skeletal muscle FFA uptake (p<0.01 for all). FFA uptake was not significantly different between more and less active twins in subcutaneous fat (0.13±0.8 vs. 0.23±1.19 μmol/100 g/min, p=0.19), visceral fat (0.20±0.15 vs. 0.26±0.18 μmol/100 g/min, p=0.38), or in skeletal muscle (0.28±0.18 vs. 0.38±0.23 μmol/100 g/min, p=0.3), but it was lower in the liver in the more active twins as compared to the less active brothers (5.5±4.3 vs. 9.0±6.1 μmol/100 ml/min, p=0.04).

Conclusion: Without the confounding effects of genetic factors, moderately increased physical activity and aerobic fitness decreases visceral and subcutaneous adiposity in normal-weighted humans. Adipose tissue FFA uptake is higher in visceral than subcutaneous adipose tissue in the fasting state and not changed by exercise. In contrast, liver FFA uptake decreases by regular exercise, and this positive effect is observed even without changes in serum FFA concentrations.

0523

Comparison of the effect of pioglitazone and insulin on postprandial lipoprotein composition and expression of genes affecting cholesterol absorption, synthesis and chylomicron assembly

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Background and Aims: Abnormal postprandial lipoproteins may be an important cause of the increased atherosclerosis found in diabetes. Both insulin and pioglitazone have been shown to reduce postprandial lipoproteins but the mechanism may be different since the thiazolodinediones increase insulin sensitivity particularly in the liver. A recent human study has demonstrated that insulin sentisation with pioglitazone has a major effects in restoring postprandial lipaemia to normal. We have previously shown altered expression in diabetes of genes involved in cholesterol absorption and synthesis and lipoprotein assembly. Niemann Pick C1-like1(NPC1L1) which facilitates cholesterol absorption and 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCoA reductase) the rate limiting enzyme for cholesterol synthesis were increased in diabetes as was microsomal triglyceride transfer protein (MTTP) which assembles the chylomicron and VLDL particles while the ATP binding cassette proteins (ABC) G5 and G8 which re-excrete absorbed cholesterol were reduced. The aim of the study was to examine the effect of insulin compared to pioglitazone on expression of genes in liver and intestine which are involved in cholesterol homeostasis in order to explain the mechanism whereby insulin sensitisation with pioglitazone restores postprandial lipaemia to normal.

Materials and Methods: Thirty zucker diabetic fatty fa/fa rats were examined. Animal experiments were carried out under licence from the Department of Health in Dublin. Diabetes was confirmed with a blood sugar>7.5. Ten of the diabetic rats were treated with insulin and 10 with pioglitazone for 2 weeks prior to saccrifice. Blood was collected at sacrifice and chylomicron and very low density lipoproteins (VLDL) isolated by sequential ultracentrifugation. mRNA expression of HMGCoA, MTTP, NPC1L1 and ABC G5 and G8 were determined in liver and intestine using real-time PCR.

Results: Blood glucose for untreated rats was 9.7±1.7 mmol/l this was significantly greater (p<0.005) than both insulin treated (7.0±1.4) and pioglitazone treated (7.9±0.4 mmol/l) which were not significantly different from each other. While both insulin and pioglitazone significantly reduced chylomicron and VLDL cholesterol, triglyceride, apo B48 and apo B100 (p<0.001) the levels in pioglitazone-treated animals significantly lower than those in the insulin-treated rats (p<0.0001). Insulin and pioglitazone significantly reduced MTTP and in both liver (p<0.001) and intestine (p<0.001) and they both increased ABCG5 and G8 in the liver (p<0.0001). Insulin significantly reduced hepatic HMGCoA reductase. In comparison to insulin pioglitazone significantly reduced hepatic, MTTP (0.0001) and NPC1L1 mRNA (Insulin 15+3 vs 8+3 p<0.0001) and significantly increased hepatic ABCG5 (21+6 vs 73+29) and G8 mRNA (20+4 vs 63+6 p<0.0001).

Conclusion: Although both insulin and pioglitazone reduced chylomicron and VLDL, pioglitazone was more effective for a similar decrease in blood sugar. This was due at least in part to the stronger effect of pioglitazone on

mRNA of genes regulating cholesterol absorption and re-excretion in the liver but not the intestine and the resulting reduction in MTP. The study explains, at least in part, the improvement in postprandial lipaemia by the addition of pioglitazone to insulin treatment.
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0524

Triglyceride disposal and lipoprotein lipase-mediated free fatty acid spillover in the splanchnic bed of dogs

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Background and Aims: The action of lipoprotein lipase (LPL) on circulating triglyceride-rich lipoproteins generates fatty acids, some of which are known to "spill over" into the systemic circulation and contribute to the free fatty acid (FFA) pool. The contribution of the splanchnic bed, and the relative contribution of the liver and nonhepatic splanchnic tissues to this process, is not known. However, the liver is not generally believed to contain LPL. We have previously shown that a commercial lipid emulsion labeled with [^{3}H] triolein is a suitable surrogate for the study of chylomicron triglyceride metabolism, and that the production of [^{3}H] oleate therefrom can be quantified to estimate spillover. The present study was conducted to determine the efficiency of triglyceride storage in the splanchnic bed and the contribution of the splanchnic bed to whole-body triglyceride disposal.
Materials and Methods: Sampling catheters were surgically placed in the femoral artery, portal vein and hepatic vein of mongrel dogs (n=5). At the same time, Transonic flow probes were placed for subsequent measurement of hepatic artery and PV plasma flow. Two weeks after the procedure, the animals were studied under postabsorptive conditions. Infusions of tracer amounts of [1-^{14}C] oleate (~0.2 μCi/min) and [9, 10-^{3}H] triolein (~1.4 μCi/min) were started, allowing 90 min to establish isotope equilibrium. Blood was taken from the three sampling sites every 7.5 min X5 for measurement of triglyceride radioactivity as well as the concentration and specific activity (SA) of plasma oleate.
Results: Plasma oleate concentration was higher in the portal vein than in the artery (449±28 vs 382±29 μmol/L, P<0.005), whereas it was lower in the hepatic vein (351±17 μmol/L) was lower than in the portal vein, P<0.005. Plasma ^{14}C oleate SA was lower in the portal vein than in the artery (1.63±.16 vs 1.88±.19 dpm/nmol, P<0.005), but the hepatic venous value (1.66±.17 dpm/nmol) was similar to that in the portal vein. Plasma ^{3}H oleate SA was similar in the artery and portal vein (6.33±.31 vs 6.00±.17 dpm/nmol, P=NS), whereas the value in the hepatic vein (8.10±.50 dpm/nmol) was higher than the PV value, P<0.005. Splanchnic uptake of the ^{3}H triglyceride was 32±7% of systemic triglyceride disappearance. Of the ^{3}H triglyceride uptake in the splanchnic bed, 26±5% occurred in nonhepatic tissues, and 74±5% in the liver. Fractional spillover of ^{3}H oleate was 59±11% and 40±9% in nonhepatic splanchnic tissues and liver, respectively. The splanchnic bed accounted for 15±3% of systemic oleate uptake and 8.7±2.0% of systemic oleate appearance.
Conclusion: These results indicate that the splanchnic bed makes a major contribution to systemic triglyceride disposal, and that the majority of splanchnic triglyceride uptake occurs in the liver. Significant spillover of fatty acids produced by triglyceride hydrolysis was observed in both the liver and in nonhepatic tissues. The enzymatic mechanism for triglyceride hydrolysis in the liver is not clear. The relevance of these observations to the fed state will require further study.
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0525

Interaction of peroxisome proliferator-activated receptors with acyl-CoA binding protein in transgenic rats

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Background and Aims: The metabolic syndrome (MetS) is characterized by central obesity, dysglycemia, dyslipidemia and/or elevated blood pressure. A central feature of the syndrome are elevated free fatty acids converted into acyl-CoAs in the cytosol and bound to storage proteins like acyl-CoA binding protein (ACBP). ACBP has previously been shown to affect insulin secretion and gene regulation. Important molecular targets for the treatment of the MetS are peroxisome proliferator-activated receptors (PPARs) – lipid sensors regulating transcription of genes involved in lipid and glucose metabolism, and inflammation. Fatty acids are natural ligands of PPARs, whereas acyl-CoA esters act as antagonists and ACBP has been demonstrated

to have a negative role in ligand-dependent PPAR transactivation *in vitro*. To determine the interaction of these two regulators *in vivo* under various physiological conditions we created a transgenic rat line over expressing mouse ACBP gene.

Materials and Methods: A rat line overexpressing the mouse ACBP gene was produced by standard techniques. Tissue levels of different acyl-CoAs were analysed by HPLC-ESI-MS/MS. Gene expression analysis was performed by quantitative RT-PCR from liver and adipose tissues under fasted or fed conditions. In addition, tg and wt rats were treated with PPAR-α agonist fenofibrate (100 mg/kg/d gavage) for 3,5 days or received diets with either long-chain (LC-diet; palm oil) or medium-chain (MC-diet; synthetic medium chain triglycerides) fatty acids for 1 month.

Results: RT-PCR and Western blot analysis demonstrated ACBP overexpression. Furthermore, in tg rats acyl-CoA levels were significantly elevated in adipose tissue. In fed state, PPAR-γ and PPAR-δ gene expression levels in tg livers compared to wt rats were significantly reduced by 82% or 62%, respectively, whereas PPAR-α expression was not affected. In adipose tissue of tg rats, both PPAR-γ and PPAR-δ were downregulated by 23% or 43%, respectively. Fasting upregulated PPAR expression (compared to fed state) in livers of tg rats (7x PPAR-α, 7x PPAR-γ, 2,2x PPAR-δ) and 3x PPAR-δ in adipose tissue, while PPAR-γ was reduced but not signifcantly. Intragastric application of fenofibrate significantly stimulated ACBP expression (4–5x) in the liver. Neither MC-diet nor LC-diet affected PPAR expression in liver of wt rats. In contrast, tg rats had reduced PPAR expression levels in liver in response to MC-diet (58%, 75%, and 50% for PPAR-α, PPAR- γ and PPAR-δ, respectively), but not to LC-diet. In adipose tissue, MC-diet induced PPAR-γ expression 63% of wt, but did not affect tg rats. Both diets reduced PPAR-δ expression of tg animals in adipose tissue (55% and 27% for LC- and MC-diet, respectively).

Conclusion: Our results demonstrate that overexpression of ACBP gene elevates acyl-CoA levels and downregulates the expression of PPARs. This downregulation was reversed by fasting. In addition, diets affected PPAR levels of tg animals differently compared to wt littermates. Hence we conclude that ACBP might act as a negative regulator *in vivo*.

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0526

Relationship between oxidized low density lipoprotein and insulin resistance in type 2 diabetes mellitus

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Background and Aims: Both low-density lipoprotein oxidation (ox-LDL) and insulin resistance (IR) play important role in the endothelial dysfunction and atherosclerosis. Recent studies have demonstrated that the amount of ox-LDL in plasma is correlated with IR in non-diabetic population. The aim of this study was to evaluate correlation of ox-LDL and IR in type 2 diabetic patients, and to compare it with normal individuals.

Materials and Methods: A total of 150 individuals aged 40 to 65 years were studied including 81 type 2 diabetic patients and 69 non-diabetic sex and BMI matched healthy persons. Exclusion criteria included smoking, treatment with lipid lowering and antihypertensive drugs, positive history of cardiovascular and renal disease. Demographic characteristics and anthropometric data of participants were recorded. Levels of circulating ox-LDL were measured with 2 monoclonal antibody-based competition ELISA. Oxidized LDL antibodies (OLAB), glucose, insulin, and HbA1C were also determined in fasting blood samples. Insulin resistance was estimated according to homeostasis model assessment of insulin resistance (HOMA-IR).

Results: In diabetic patients ox-LDL concentration was significantly lower than normal controls (11.8±5.6 mU/l vs. 15.7±6.9 mU/l $p<0.001$). The difference in OLAB concentration in the two groups were not statistically significant. HOMA-IR was 2.77±1.96 in diabetic patients and 1.41±0.91 in controls ($p<0.001$). There was a significant correlation between ox-LDL with total ($r=0.33$, $p<0.005$) and low density cholesterol ($r=0.34$, $p<0.005$) in both patients and controls. After considering all the relevant factors including age, duration of diabetes, BMI, systolic and diastolic blood pressure, serum lipids, and HOMA-IR, regression analysis demonstrated that there was a significant correlation between ox-LDL and HOMA-IR in patients ($r=0.39$, $p<0.005$) but not in healthy controls. OLAB had weak but significant correlation with BMI in both diabetic and non diabetic groups($r=0.23$ and $r=0.24$ respectively, $p<0.05$).

Conclusion: Our results suggest an association between insulin resistance and increased LDL oxidation independent of the effect of other contributing factors.

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0527

The impaired regulation of LPL in adipose tissue contributes to the hypertriglyceridaemia in insulin resistance

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Background and Aims: Insulin resistance (IR) is associated with the disturbances in the metabolism of both glucose and lipids. In hereditary hypertriglyceridemic (HHTg) rats, the non-obese model of IR, the diet with high content of sucrose induces hypertriglyceridemia, elevation of serum FFA and substantial resistance of glucose metabolism to insulin action. The postprandial LPL activity is considered to be the rate-limiting factor determining the clearance of circulating triglycerides (Tg) but no less important is the ability of adipose tissue to store released FA as Tg via their esterification. The purpose of this study was to determine whether the postprandial modulation of LPL activity in adipose tissue may contribute to the elevated triglyceridemia in HHTg rats with resistance of glucose metabolism to insulin action induced by HS feeding.
Materials and Methods: Control (C) and HHTg rats (weight 300±20 g) were fed either standard (SD) or high sucrose (HS, 70% of calories as sucrose) diet for two weeks. LPL activity was determined in the homogenates from distal parts of adipose tissue by the release of fatty acids from labeled substrate (^{3}H-trioleine). The sensitivity of glucose metabolism to insulin in adipose tissue was determined by ^{14}C-glucose incorporation into lipids *in vitro*.
Results: The increased supply of sucrose induced in HHTg significantly higher postprandial insulinemia than in control rats (780±29 vs 440±23 pmol/l). The HHTg rats exhibited elevated postprandial serum Tg compared to controls both on SD (by 180%) and HS diet (by 290%). The effect of insulin on *in vitro* glucose incorporation to Tg was similar in C and HHTg group on SD but HS feeding led in these groups to opposite effects - the insulin sensitivity in HHTg group decreased, in control rats it was potentiated. The postprandial LPL activity was lower in HHTg than in C rats on SD ($p<0,01$) but HS feeding led to its significant increase ($p<0,01$). The LPL activity in control group tends to be lowered by the diet ($p=0,06$). The activity of adipose tissue LPL of control rats decreased in fasting but in HHTg group this regulation was blunted and the LPL activity remains high what indicates the impairment of the regulation of synthesis and/or the activation of LPL. We found strong correlation ($r^2=0,72$) between postprandial serum Tg content and adipose tissue LPL activity in HHTg rats both on standard and HS diet.
Conclusion: In contrast to controls, in HHTg rats HS feeding leads to the increase in LPL activity in adipose tissue probably as the result of higher insulinemia. In spite of higher LPL activity in HHTg groups the serum Tg content does not drop but significantly increased. We suppose that the impaired ability of HHTg adipose tissue to uptake glucose and to incorporate it into intracellular triglycerides leads to the increased escape of FFA derived from circulating lipoproteins by LPL. The released fatty acids remain in the circulation and are turned in liver into VLDL that contributes to the maintenance of hypertriglyceridemia.
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0528

Small dense LDL and oxidized LDL: differences between type 2 diabetes and familiar combined hyperlipidaemia

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Background and Aims: Type 2 diabetes and combined hyperlipidemia (CH) are associated with premature atherogenesis. In both diseases total and LDL cholesterol (LDL-C) are elevated as well as level of triglycerides. Obesity and insulin resistance are also present in both disorders. The present study analyze relationship between LDL particle size and lipids level, level of LDL oxidation, CRP and insulin sensitivity in diabetics and in patients with CH.

	C/SD		C/HS		HHTg/SD		HHTg/HS	
	fasting	*fed*	*fasting*	*fed*	*fasting*	*fed*	*fasting*	*fed*
serum Tg mmol/l	0,8±0,05	0,9±0,1	0,7±0,1	1,35±0,2	0,8±0,15	2,5±0,3	1,36±0,2	5,3±0,2
LPL activity umol FA/g/h	4,5±0,3	9±0,5	3±0,4	6,9±0,45	3,7±0,3	3,5±0,25	5,4±0,6	6,9±0,7
	−insulin	***+Insulin***	***−insulin***	***+insulin***	***−insulin***	***+insulin***	***−insulin***	***+insulin***
glucose incorporation into Tg umol/g/h	1,3±0,12	1,6±0,13	1,6±0,1	3,2±0,3	1,7±0,06	2,1±0,09	1,2±0,09	1,9±0,08

Materials and Methods: Investigation was performed in 60 type 2 diabetics: 27 without (group A) and 33 with dyslipidemia (group B), and in 62 non diabetics: 28 patients without dyslipidemia (group C) as well as in 34 patients with CH (group D). LDL size was measured by gradient gel electrophoresis, cut-off values was diameter <25, 5 Å. Oxidized LDL (ox-LDL) was measured by ELISA methods (Mercodia). Total cholesterol, HDL cholesterol (TC, HDL-C) and triglyceride (TG) were determined by chromatography, LDL-C and non HDL-C was calculated. Apolipoproteins, Lp (a) and CRP were measured by nephelometric methods. Insulin was assessed by RIA methods and insulin sensitivity was estimated by HOMA-IR.
Results: In our diabetics, 32 of all (62, 6%) had small dense LDL particle, but there was no significant differences between groups A and B. In group B patients had higher TC, LDL-C, non HDL-C, TG ($p<0.01$), CRP ($p<0.05$). Levels of HDL-C and Lp(a) were similar in both groups, as well as HOMA-IR. Ox-LDL was higher in group B (117, 12 vs. 91, 85 IU/L, $p<0.01$). In both diabetic groups LDL particle size positive correlated with HDL-C, Apo AI, AII ($p<0.05$), but negative correlated with levels of TG, Apo E and HOMA-IR ($p<0.05$). The level of Ox-LDL correlated with TC, LDL-C, non HDL-C and Apo B 100 ($p<0.001$), as well as al level of TG, Apo AI, ($p<0.05$). In non diabetics, CH patients only 17 out of 62 (25, 9%) had small dense LDL particles. Patients from group D had higher TC, LDL-C, non HDL-C and TG ($p<0.01$). There was no significant differences in levels of HDL-C, Lp (a), but Apo B 100 and Apo E were higher in group D ($p<0.01$). We have not found significant difference in the LDL particle size in these groups (26, 48 vs. 26, 46 Å, p=NS), but dyslipidemic patients had higher ox-LDL levels (111, 83 vs. 99, 72 IU/L, $p<0.05$). Also, in non diabetic groups insulin sensitivity was similar. LDL particle size in all non diabetics positive correlated with TC ($p<0.05$) and with Lp (a) ($p<0.01$). In these patients ox-LDL correlated with TC, LDL-C, non HDL-C and TG ($p<0.01$).
Conclusion: Diabetics predominantly have small dense LDL. LDL particle size negative correlate with insulin resistance (HOMA-IR) and triglyceride but positive with HDL-C, Apo AI and AII. In those patients with dyslipidemia oxidation of LDL was higher, but there was no correlation between small dense LDL particle size and ox-LDL. In contrast, CH patients despite non dominantly small dense LDL, had higher level of LDL oxidation.
Supported by Mercodia

0529

Free fatty acids as mediators of adaptive compensatory responses to insulin resistance induced by dexamethasone treatment in rats

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Background and Aims: We previously showed that chronic low-dose dexamethasone (DEX) treatment in rats induces insulin resistance with compensatory hyperinsulinaemia and reduction in food intake. Here we test the hypotheses that DEX-induced hyperlipacidaemia mediates both insulin resistance and insulin hyperproduction and that a rise in circulating leptin is responsible for the anorectic effect of DEX.
Materials and Methods: DEX treatment (0.125 mg/kg/day dexamethasone phosphate s.c.) of Sprague-Dawley rats for 20 days; transient reduction of plasma free fatty acid (FFA) levels by repeated i.p. injections of the antilipolytic agent nicotinic acid, prior to intravenous glucose tolerance test and intraperitoneal insulin tolerance test; monitoring of plasma glucose, FFA, insulin and leptin levels during DEX treatment; evaluation of leptin expression by Northern blot in white adipose tissue (WAT) at the end of DEX treatment.
Results: Upon DEX treatment, an early increase in circulating FFA followed by hyperinsulinaemia was observed, associated with normal or even slightly decreased glycaemia. Reduction in food intake and impairment in body weight gain was confirmed to occur. A transient pharmacological reduction of elevated circulating FFA reversed the DEX-induced insulin resistance and the compensatory post-loading hyperinsulinaemia, supporting the hypothesis that the chronic elevation in FFA is the common mediator of these changes. Despite enhanced leptin expression in WAT, DEX-treated rats showed no significant increase in plasma leptin levels, thus suggesting that the anorectic effect of DEX should be mediated, at least partially, by some other factor. Based on the reported anorectic effect of central FFA administration, we postulate that the chronic elevation of circulating FFA might be involved in the limitation of food intake in DEX-treated rats, probably acting through the accumulation of long chain fatty-acylCoAs in selective nutrient-sensing neurons.

Conclusion: Our results support the idea that during DEX treatment chronic elevation of plasma FFA plays an important role in the adaptive regulation of both insulin action and energy balance, not only by potentiating insulin secretion, but also by providing a neuronal signal of "nutrient abundance" capable of restraining feeding behaviour.
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0530

A supervised exercise programme increases HDL ApoA-1 production rate in type 2 diabetes

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Background and Aims: Dyslipidaemia in type 2 diabetes is characterized by increased plasma triglycerides and decreased HDL cholesterol. Research indicates that the dyslipidaemia and insulin resistance of type 2 diabetes can be improved by aerobic exercise. Although patients are advised to exercise as a part of their therapy, unless this is part of a supervised exercise program this is usually unsuccessful. This study has measured the effect of exercise on HDL apoA-1 kinetics in two groups of patients following a 6 month exercise plan. One group had weekly meetings with an exercise trainer (supervised), the other group did not see an exercise trainer after their initial contact session (unsupervised).
Materials and Methods: Ten moderately obese type 2 diabetic patients who had failed to show improved glycaemic control with diet and tablets (HbA1c>7.5%) participated in this study. Those on oral hypoglycaemic agents did not change treatment throughout the study. All patients were given an initial training session and an individualised exercise programme and were asked to exercise for 20–40 min at 60–85% of maximal oxygen uptake (VO_{2max}) four times per week, in a mode of aerobic activity of their choice, for a period of 6 months. Patients were then randomised into a supervised (n=6; BMI 28.54±1.75 kg/m^2, age 57.8±3.6 years, 4F:2M) or unsupervised (n=4; BMI 32.6±1.83 kg/m^2, age 54.8±6.0 years, 1F:3M) group. HDL apoA-I metabolism was measured with an infusion of 1-^{13}C leucine for 9 hours before and after the exercise programme. Patients did not exercise for 3 days prior to the metabolic study day. HDL was isolated by ultracentrifugation. ApoA-I enrichment was measured after separation from other apolipoproteins by polyacrylamide gel electrophoresis. ApoA-I concentration was measured by immunoturbidimetry. Insulin sensitivity was calculated by HOMA. The fractional secretion rate (FSR) of HDL apoA-I was calculated using a regression model. HDL apoA-I production rate (PR) was calculated from the product of the FSR and the pool size. The steady state enrichment of α-ketoisocaproate was used as an estimate of the precursor pool enrichment. Results are presented as mean±SEM.
Results: Those in the supervised exercise group showed a significant decrease in % HbA1c (8.47±0.47 v 7.45±0.51; p<0.005) and plasma triglyceride (1.06±0.23 v 0.8±0.2 mmol/l; p<0.03), an increase in insulin sensitivity (HOMA: 7.39±1.39 v 3.69±0.29; p<0.04). There was an increase in HDL cholesterol (1.05±0.09 v 1.34±0.10 mmol/l; p<0.03), apoA-1 concentration (1.18±0.12 v 1.42±0.11 g/l; p<0.05) and a significant decrease in HDL triglyceride (0.16±0.02 v 0.10±0.01 mmol/l; p<0.05). The increase in HDL was due to an increase in apoA-1 PR (5.99±0.75 v 8.79±1.04 mg/kg/day; p<0.03). There was no significant change in HDL apoA-1 FSR (0.15±0.01 v 0.18±0.02 pools/day). The unsupervised group showed no significant changes in any of the parameters measured.
Conclusions: The study suggests that the increase in HDL cholesterol following the supervised exercise programme was due to an increase in HDL production rate. This may be due to an improvement in insulin sensitivity which was not apparent in the unsupervised group.
Supported by BHF

PS 31

Cardiac metabolism

0531

Cardiac pyruvate dehydrogenase kinase (PDK) 4 is enhanced by increased LXR signaling in the heart

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Background and Aims: The liver X receptor (LXR) subfamily of nuclear receptors is activated upon binding of ligands, which probably comprise oxidised cholesterol derivatives. Improved glucose tolerance and insulin sensitivity in response to LXR activation has been reported in genetic and dietary models of type 2 diabetes: this may reflect decreased hepatic glucose output secondary to an LXR-led reduction in gluconeogenic capacity as LXR activation represses expression of hepatic gluconeogenic

enzymes. However, activation of LXRs also causes a dramatic increase in hepatic triglyceride production and both increased lipid delivery and/or inappropriate induction of fatty acid synthesis in extrahepatic tissues could impair insulin action through excessive tissue lipid accumulation. The heart is particularly susceptible to adverse effects of lipid accumulation in diabetes. Increasing glucose oxidation and decreasing fat oxidation during cardiac hypertrophy and during reperfusion of ischemic hearts is believed to be beneficial in maintaining cardiac contractility and efficiency. Glucose oxidation is constrained by the activity of the pyruvate dehydrogenase kinases (PDKs), which inactivate pyruvate dehydrogenase complex (PDC) by phosphorylation. Cardiac PDK isoform 4 (PDK4) expression is up-regulated in diabetes, resulting in PDC inactivation.

Materials and Methods: Male mice (4–6 months old, 25–30 g) were allowed food and water ad libitum. TO901317 (0.01%) was administered as a component of the diet for 5 days. Animals were killed in the absorptive state and hearts removed for analysis of PDK4 protein expression and triglyceride (TAG) content.

Results: LXR activation *in vivo* by the provision of TO901317 as a component of the diet (5 days, 0.01%) causes a marked (3.6-fold; $P<0.05$) enhancement of cardiac PDK4 protein expression, in conjunction with cardiac TAG accumulation (a 3.8-fold increase) in mice. Protein expression of PDK1 and PDK2 was unchanged by LXR activation.

Conclusion: As the heart has an optimal balance between the use of lipid and glucose as oxidative substrate, altering this balance through activation of LXR and increased PDK4 protein expression, despite improved insulin action, might be detrimental to cardiac function in diabetes.

Supported by Diabetes UK

0532

Over-expression of CYP2J2 specifically augments cardiac pyruvate dehydrogenase kinase 4 expression

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Background and Aims: Epidemiological studies reveal that people with diabetes and hypertension are at higher risk for the development of cardiovascular disease. The heart in uncontrolled diabetes is constrained from switching to glucose oxidation, and relies almost exclusively on lipid as an energy source. Peroxisome proliferator-activated receptor (PPAR)-α is a nuclear receptor involved in regulation of lipid metabolism and the PPARα/PGC-1α axis is activated in the diabetic heart. Cardiac-specific over-expression of PPARα results in enhanced sensitivity to ischemic injury and recapitulation of many of the metabolic abnormalities of the diabetic heart, including decreased glucose oxidation. The expression of cardiac pyruvate dehydrogenase isoform 4 (PDK4), a PPARα target gene, is up-regulated in diabetes, resulting in pyruvate dehydrogenase complex (PDC) inactivation by phosphorylation and suppressed glucose oxidation. The expression of PDK1 is augmented by the HIF-1 transcription factor, which drives hypoxic gene expression. The biologically active cis-epoxyeicosatrienoic acids (EETs) that regulate blood pressure, and can activate PPARα *in vitro*, are products of CYP2J2, an abundant epoxygenase in human cardiovascular systems. We investigated whether modification of CYP2J2 activity influences the cardiac PDK isoform profile.

Materials and Methods: Female cardiac-specific CYP2J2 transgenic mice and wild type controls (C57 black, 4–6 months old, 25–30 g) were allowed food and water ad libitum or fasted for 24 h. Animals were then killed and hearts and kidneys removed for analysis of PDK4 protein expression in comparison to that of PDK1. Anti-PDK1 ann PDK4 antibodies, generated in rabbits against recombinant PDK4 protein, were generously provided by Professor Bob Harris (Indianapolis, U.S.A.).

Results: Upon fasting, PDK4 protein expression increased (3.7-fold; $P<0.01$) in wild type mice compared to fed animals. This response was augmented (by2-fold; $P<0.01$) in the hearts, but not kidneys, of CYP2J2 transgenic mice. Fasting had no significant effect on the levels of PDK1 in either wild type or CYP2J2 transgenic mice.

Conclusion: Thus, over-expression of CYP2J2 specifically augments cardiac PDK4 expression suggesting that the generation of EETs may facilitate PDK4 expression in heart, but not kidney, but that PDK1 is not a target for CYP2J2-generated compounds.

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0533

Both subcutaneous and epicardial adipose tissue is a significant source of proinflammatory factors in cardiac surgery patients: possible role in postoperative hyperglycaemia and insulin resistance

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Background and Aims: Critically ill patients frequently develop hyperglycemia and insulin resistance. Its detailed etiopatogenesis is still only partially understood. Here we studied the role of adipose tissue hormonal production in the development of postoperative insulin resistance in elective cardiac surgery patients.
Materials and Methods: Serum concentrations, subcutaneous (s.c.) and epicardial (epi.) adipose tissue mRNA expression of interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-α (TNF-α), leptin, resistin and adiponectin were measured in 10 patients with elective cardiac surgery operation using commercial Lincoplex kits and real time PCR, respectively. Adipose tissue samples were taken at the beginning and at the end of operation. Blood samples were withdrawn before, at the end and 6, 12, 24, 48 and 120 hours after the end of operation, respectively.
Results: The rate of insulin infusion required to maintain euglycemia increased up to 8-fold 12 hours after the operation indicating the development of insulin resistance. Serum IL-6 levels increased 43-fold and peaked 12 hours after operation. MCP-1 peaked 6-fold at the end of operation, while less pronounced peaks of TNF-α and leptin, 6 and 20 hours after operation, respectively were detected. Circulating resistin levels increased 2.7-fold 6 hours after operation and remained 3.5-fold elevated until 48 hours after operation. In contrast adiponectin levels were not significantly affected by the operation. TNF-α and resistin mRNA expression increased markedly during the operation in s.c. adipose tissue. IL-6 and MCP-1 mRNA expression increased in both s.c. and epi. adipose tissue. Neither leptin or adiponectin mRNA expression were significantly affected by the operation.
Conclusion: Elective cardiac surgery operation markedly activates production of proinflammatory cytokines in both subcutaneous and epicardial adipose tissue. We suggest that this production may significantly contribute to the development of postoperative insulin resistance in these patients.
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0534

Short-term exposure to polyunsaturated high fat diet preserves cardiac contractile function in rats

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Background: Cardiovascular complications are common in type 2 diabetes. Previously, we demonstrated cardiac functional, structural and molecular changes in Wistar rats after long-term exposure to high (saturated) fat diet (HSFD), resembling diabetic cardiomyopathy (DCM). The current study tests the hypothesis that polyunsaturated fatty acids (PUFA) may protect against HSFD-induced cardiac changes in rats, following short-term exposure.
Methods: Male Wistar rats (bodyweight, BW; 269 g± 14,0 g) received HSFD (n=11), PUFA-enriched diet (PUFAD; n=11), or a low fat diet (LFD; n=11) for four weeks. At day 25, all rats were subjected to an oral glucose (2 g/kg BW) tolerance test. Post-load blood glucose (BG) levels were determined at t=0, 15, 30, 60, 90, 120 min. At day 27, echocardiography was performed to determine left ventricular end diastolic and end systolic wall thickness, lumen diameters, fractional shortening (FS%) and epicardial thickness (EPI). At day 28, rats were decapitated and plasma insulin, non-esterified fatty acids (NEFA) and triglycerides (TG) levels were determined in trunk blood.
Results: Body weight and heart-to-tibia ratio were similar in the 3 groups. Fasting BG did not differ among groups, while post-load BG was increased in HSFD-treated rats at 60 min [LFD 8,5 vs. HSFD 10,3, $p<0.05$] and at 90 min [LFD 7,2 vs. HSFD 8,9 $p<0.05$]. Post-load BG at 60 and 90 min was comparable in HSFD- and PUFAD-treated rats. Reduced plasma NEFA and TG concentrations were observed in HSFD- and PUFAD-treated rats, relative to LFD-controls, whereas similar insulin levels and HOMA-IR were observed in all groups. Echocardiography revealed a significant decrease in FS% in HSFD-rats, whereas no difference was found in PUFAD-treated rats, compared to LFD-controls [LFD: 48,9% vs. HSFD: 42,0% vs. PUFAD: 48,7%, $p<0.05$ One-way ANOVA]. Both HSFD- and PUFAD-fed rats had increased EPI, as compared to LFD-rats [LFD: 0,05 cm vs. HSFD: 0,08 cm vs. PUFA: 0,07 cm].
Conclusions: A short-term exposure to a HSFD resulted in reduced glucose tolerance, contractile dysfunction and increased epicardial thickness, resembling DCM. Interestingly, PUFAD preserved cardiac contractile function, in spite of the presence of metabolic changes similar to those observed in HSFD-treated rats. The molecular mechanisms underlying the cardiac protective effects of PUFAD remain to be investigated.
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0535

Abnormal left ventricular energy metabolism in obese men with preserved systolic and diastolic functions is associated with insulin resistance

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Background and Aims: It has been hypothesized that perturbations in cardiac energy metabolism might be among the earliest alterations in diabetes preceding functional and pathological changes. This study evaluated left ventricular (LV) structure and function in relation to energy metabolism and cardiovascular risk factors in overweight/obese men using magnetic resonance (MR) techniques.
Materials and Methods: We studied 81 healthy men (age range: 22–55 years, body mass range: 19–35 kg/m^2) by means of cardiac MR imaging (MRI) and ^{31}P-MR spectroscopy (MRS) in the resting and fasted conditions and stratified them in quartiles of BMI (cut offs: 23.2, 25.5 and 29.0 kg/m^2).
Results: The morphological features showed that the LV mass increased across increasing quartiles of BMI (r=0.39; P<0.002) meanwhile the chambers volume did not differ. Parameters of LV systolic (ejection fraction, stroke volume) and diastolic function (early/atrial peak ratio and deceleration time) were not different among quartiles. In contrast the phosphocreatine (PCr)/ATP ratio was reduced across increasing quartiles of BMI (2.25±0.52, 1.89±0.26, 1.99±0.38 and 1.79±0.29; P<0.006) in association with insulin sensitivity (calculated using the updated computer HOMA2 model based on fasting plasma glucose and insulin concentrations) and this relation was independent of age, BMI, blood pressure, end diastolic wall mass, serum HDL-cholesterol, triglycerides, smoking habits and metabolic syndrome.
Conclusion: Abnormal LV energy metabolism is detectable in obese men in presence of normal LV function and these data support the hypothesis that metabolic remodeling in insulin resistant states precedes the functional and structural remodeling of the heart before the potential onset of frank hyperglycemia.
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0536

In wild type mice long-term exposure to high-fat diet induces systemic insulin resistance without affecting cardiac function

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Background and Aims: Cardiac dysfunction is prevalent in diabetes mellitus type 2 (DM2), and in time, may progress from mild functional changes into overt heart failure. This study aims to reveal cardiac changes following 20 weeks exposure to high fat diet in wild type C57Bl/6J mice.
Materials and Methods: Sixteen male mice (17.0 g±0.9 g) received either a high fat (HFD; 24% fat; n=8) or a low fat diet (LFD; 4% fat; n=8) diet. Fasting blood glucose (FBG) was determined each 2 weeks. After 18 weeks of diet, mice underwent an oral glucose (2 mg/g) tolerance test. BG levels were determined at t=0, 15, 30, 60, 90, 120 min after the glucose load. In week 20, left ventricular (LV) end diastolic (EDD) and systolic diameters (ESD) and fractional shortening (FS%) were measured by echocardiography. At decapitation, plasma insulin, non-esterified fatty acids and triglycerides were determined in trunk blood.
Results: At 20 weeks, HFD- vs. LFD-mice had higher body weight (38.6 g vs. 31.9 g, p<0.05), but similar heart-to-tibia ratio's. In HFD-mice, FBG tended to increase from week 14 on (p<0.05). Post-load BG was increased in HFD- vs. LFD-mice at 30 min and at 90 min (both p<0.05). In HFD- relative to LFD-mice, plasma insulin concentrations were increased 2.4-fold (p<0.05). HFD- vs. LFD-mice tended to be insulin resistant, as assessed by HOMA-IR (p=0.05). Cardiac parameters, including LVEDD, LVESD and FS% were similar in both groups, however, HFD-mice showed increased epicardial fat pad (0.07 vs. 0.05 cm; p<0.05).
Conclusion: Long-term exposure to HFD in wild type C57Bl/6J mice resulted in a DM2 phenotype, including obesity, hyperglycemia and insulin resistance, without affecting cardiac function. These data suggest that C57Bl/6J mice may be resistant to HFD-induced cardiac dysfunction or that cardiac abnormalities may be a late event in HFD-related DM2 in these mice.
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0537

Cardiac dysfunction in insulin resistant high-fat diet fed rats associates with elevated FAT/CD36-mediated fatty acid uptake and esterification

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Background and Aims: Changes in cardiac substrate utilization leading to altered energy metabolism may underlie the development of diabetic cardiomyopathy (DCM). We studied cardiomyocyte substrate uptake and utilization and the role of fatty acid translocase (FAT) FAT/CD36 in relation to *in vivo* cardiac function in a non-obese rat model of high-fat diet (HFD)-induced insulin resistance.
Materials and Methods: Rats were exposed to HFD or low-fat diets (LFD) for 10 weeks. In vivo cardiac contractile function was monitored by echocardiography. Substrate uptake and utilization were determined in isolated cardiomyocytes.
Results: HFD- versus LFD-feeding induced left ventricular (LV) dilation, increased LV free wall thickness and decreased fractional shortening. Cardiomyocytes from HFD-hearts were insulin-resistant as reflected by 41% (P<0.001) and 58% (P<0.02) decreases in insulin-mediated stimulation of glucose uptake and phosphorylation of glycogen synthase kinase 3, respectively. Fatty acid uptake was 1.4-fold increased in HFD- versus LFD-cardiomyocytes (P<0.001), which could be ascribed to an increased abundance of FAT/CD36 at the sarcolemma rather than alterations in FAT/CD36 protein levels. Finally, in HFD-cardiomyocytes, fatty acid oxidation rates were decreased by 22% (P<0.01), whereas fatty acid esterification into triacylglycerols and phospholipids were increased by 1.4- and 1.5-fold, respectively (both P<0.05).
Conclusion: HFD-feeding induces cardiac contractile dysfunction that associates with a permanent relocation of FAT/CD36 to the sarcolemma. This maladaptive presence of FAT/CD36 at the sarcolemma contributes to enhanced rates of fatty acid uptake, myocardial triacylglycerol-accumulation and accompanying insulin resistance, and may contribute to the development of DCM.
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0538

Cardio protective effect of glucose-insulin infusion on acute digoxin toxicity in rat

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Background and Aims: Recently we observed a case of digoxin and insulin self-poisoning without cardiac repercussion. We raise the hypothesis that insulin may have a cardio-protective effect in case of digoxin toxicity. Therefore, we have evaluated the effect of glucose-insulin infusion on mortality and ECG abnormalities during acute digoxin toxicity in rats.
Materials and Methods: Before and after a hyperinsulinemia-euglycemia clamp, rats in glucose-insulin-digoxin (GID) group (n=10) received an intravenous infusion of 12 ml/h or 2,5 ml/h digoxin (0.25 mg/ml) respectively until death occured. Animals receiving digoxin or saline solution intravenously served as control (n=10). ECG recording was performed in all animals over the entire period. Serum insulin and digoxin concentrations were measured by ELISA method after digoxin administration.
Results: When digoxin was administered after the clamp, all animals in GID group were alive, whereas 80% of animals in digoxin group were dead (p<0.001) after thirty minutes. Digoxin administration provoked rapid death of rats in digoxin group in 15±12 min whereas in GID group the survival period was significantly increased to 38±3 min (p<0.001). Twenty minutes after digoxin administration, P waves disappeared for 78% of animals in digoxin group while they were present in all rats of GID group (p<0.0001). Animal death occurred after a digoxin infusion volume of 7.7 +0.6 ml and 3.0+2.4 ml in GID and digoxin group respectively (p<0.001). Five minutes after digoxin administration, potassium plasmatic level increased significantly in digoxin group as compared to GID group: 7.1±2 mmol/l vs. 4.4±0.4 mmol/(p<0.001). When digoxin was infused before the clamp, 40% of animals in GID group were alive after 180 min and the others 60% were died after 109+19 min whereas death of rats in digoxin group occured in 74+10 minutes (P<0.001). Death of animals was preceded by the P waves disappearing. Volume of digoxin infusion in GID group was twice that of the digoxin group. Thirty minutes

after digoxin administration, potassium plasmatic level increased significantly in digoxin group as compared to GID group: 6.9+0.5 mmol/L vs 4.9+ 0.3 mmol/L vs ($p<0.001$).
Conclusion: Glucose-insulin infusion delayed the abnormalities in cardiac conduction and improved rat survival after acute digoxin toxicity. These results suggest a cardioproctective effect of insulin in case of acute digoxin toxicity.

PS 32

Brain metabolism

0539

5ptase IV inhibits inositide trisphosphate accumulation in hypothalamus and regulates food intake and body weight

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Background and Aims: The lipid metabolizing enzyme, phosphatidylinositol 3-kinase (PI 3-kinase), exerts an important role in the transduction of the anorexigenic and thermogenic signals delivered by insulin and leptin to first-order neurons of the arcuate nucleus in the hypothalamus. The termination of the intracellular signals generated by the activation of PI 3-kinase depends on the coordinated activity of specific inositol phosphatases, which are poorly characterized in hypothalamus.
Materials and Methods: All studies were performed with male Wistar rats (~200 g). The investigation followed the University guidelines for the use of animals in experimental studies and conforms to the NIH publication No. 85-23 revised 1996. By sequence homology with the classical 5′- phosphatases SHIP-1 and SHIP-2 we identified the enzyme phosphoinositide-specific inositol polyphosphate 5′- phosphatase IV (5ptase IV) in hypothalamic regions involved with the control of feeding and thermogenesis. The expression, distribution and activity of this enzyme were evaluated by immunohistochemistry, immunoprecipitation, real time-PCR, *in situ* hybridizaton and knock down by antisense oligonucleotide.
Results: Phosphoinositide-specific inositol polyphosphate 5-phosphatase IV (5ptase IV) is highly expressed in neurons of the arcuate and lateral nuclei of the hypothalamus. Upon intracerebroventicular (ICV) treatment with insulin, 5ptase IV undergoes a time-dependent tyrosine phosphorylation, which follows the same patterns of canonical insulin signaling through the insulin receptor, IRS2, and PI 3-kinase. This tyrosine phosphorylation activates 5ptase IV catalytic activity towards 5′ phosphorylated inositide phosphate. To evaluate the participation of 5ptase IV in insulin action in hypothalamus, we employed a phosphorthioate modified antisense oligonucleotide specific for this enzyme. The treatment of rats with this oligonucleotide for four days reduced the hypothalamic expression of 5ptase IV by ~80%. This was accompanied by a ~70% reduction of insulin-induced tyrosine phosphorylation of 5ptase IV and by an increase in basal accumulation of phosphorylated inositols in the hypothalamus. Finally, the inhibition of hypothalamic 5ptase IV expression by the antisense approach resulted in reduced daily food intake, body weight loss and decreased 12 h spontaneous food intake.
Conclusion: 5ptase IV is a powerful regulator of signaling through PI 3-kinase in hypothalamus and may become an interesting target for therapeutics of obesity and related disorders.
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0540

HPA axis regulation reaches a new setpoint in acute and chronic type 1 diabetes: implications for brain pathology

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Background and Aims: The influence of diabetes mellitus on brain pathology is increasingly recognized. Previous contributions of our laboratory demonstrated in one month experimental type 1 diabetes (streptozotocin-STZ-treated mice) hippocampal disturbances, such as astrogliosis and increased oxidative stress.Diabetic animals also show aberrant activity of the glucocorticoid system, including increased circulating corticosterone and lack of periodicity. In our model the hypothalamus-pituitary-adrenal (HPA) axis reached a new setpoint characterized by higher circulating corticosterone. Based on these results and the involvement of the hippocampus in stress-system regulation via the HPA axis,we determined markers of HPA axis activation in acute and chronic diabetes, at 2 and 11 days of disease, respectively.
Materials and Methods: Three-months-old C57BL/6J male mice were injected with STZ (170 mg/kg ip) or vehicle.Two or 11 days after the onset of the disease the mice were sacrificed by decapitation and blood samples were taken in order to determine CORT and ACTH plasma levels. At the same time, glycemia and body, thymus and adrenal weight were measure. Brains and adrenal glands were processed for *in situ* hybridization and RT-PCR respectively.
Results: Two days diabetic mice show increased plasma corticosterone, while plasma ACTH is decreased, and AVP

mRNA in the paraventricular nucleus (PVN) of the hypothalamus is elevated. In addition, 11-days diabetic mice express less hippocampal MR mRNA compared to controls. The expressions of neither GR mRNA in the hippocampus and PVN, nor CRH in the PVN were changed in diabetic mice at any time point studied. The AVP and MR mRNA alterations in parallel with decreased ACTH, but in the face of exaggerated corticosterone secretion mark a profound disturbance in HPA axis regulation, most likely including sensitization of the adrenals to ACTH.Indeed, RT-PCR revealed the increased expression of ACTH receptors MC2 and MC5 in the adrenal glands in acute and chronic diabetes.

Conclusion: The fast upregulation of ACTH receptors in the adrenal glands is a likely mechanism that allows low plasma ACTH concentrations to trigger and maintain corticosterone hypersecretion. A better understanding of these mechanisms may explain how diabetic pathophysiology causes adaptation in the CNS leading to damage and cognitive impairment.

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0541

Differences in regional brain activation response to changes in plasma glucose according to route of administration: seeking the mechanisms of appetite control in man

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Background: Type 2 diabetes (T2DM) and obesity are lifestyle diseases, exacerbated by imbalances in matching energy intake and expenditure. Central homeostatic mechanisms maintain energy balance. We hypothesise that the cortical response to nutrient intake may also be important in regulating energy intake. Glucose is the brain's primary fuel and major biochemical disturbance in T2DM. Its neural effects when ingested, as demonstrated by our group using fMRI, reveal activation of cortical centres involved in food seeking behaviour and achieving reward. However whether this response is directly related to the rise in blood glucose *per se*, or to more generic processes of oral nutrient intake is not known. We have thus compared regional brain responses to oral intake with intravenous (IV) infusion of glucose to discriminate the neural correlates of glucose interoception from oral ingestion.

Methods: 4 healthy volunteers (median(range)age 22(19–55)yrs) having fasted overnight (8 hrs), underwent a 40minute continuous fMRI scan (gradient echo EPI, 56 axial slices, 2.5 mm thickness, TR=10s) on 2 separate occasions. During each scan subjects either drank a 75 g glucose solution or had a continuous IV glucose infusion that mimicked the rate of change in plasma concentration of the glucose ingestion. Arterialised blood samples were obtained from matched studies.

Results: MR signal changes, indicating changes in neuronal activation, were identified across whole brain in response to both stimuli with a cluster level p=0.0003. At this cluster-level we expect <1 false positive cluster per brain. Major amplitude change occurred around 10(+/−5) minutes. There was no significant difference between mean glucose concentrations of oral v IV glucose at this time (6.0v5.7 mmol/l [p=0.10]). Regions showing similar changes in signal with oral and IV glucose were located in the orbitomedial frontal cortex (OMF), anterior cingulate cortex (ACC), right insula and the amygdala/hippocampus. Changes in signal where oral glucose only occurred were in visual cortex, parietal lobe and the uncus. Changes in signal where IV glucose alone was detected were in left insula, cerebellar vermis and the sensorimotor cortex.

Conclusion: Changes in plasma glucose concentration (or the associated insulin response), activated brain regions subserving reward (amygdalae, hippocampus), hedonic value of food (OMF) and interoception (ACC). Brain regions responding to a gastric signal only were most noted in insula cortex, which is increasingly recognised to exhibit extensive regional subspecialisation to gustatory stimuli. Activations seen with IV glucose alone, perhaps indicate responses to mismatches between expectation and changes in the body's internal environment (interception). The brains response to changes in plasma glucose occurring as a result of oral ingestion or IV infusion, revealed a complex neural response involving regions controlling ingestive behaviour, hedonic value of nutrients, interoception and reward, and not solely homeostatic control centres. These data provide a firm basis for extension of these studies to people predisposed to obesity and T2DM, seeking differences that may contribute to the development of these conditions.

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0542

Pre-labeling: a novel approach towards measuring absolute glycogen concentration changes with NMR

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Background and Aims: ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy has been recently established as the only method to measure brain glycogen non invasively. However, all ^{13}C NMR studies of brain glycogen to date relied on observing the incorporation of ^{13}C label to overcome sensitivity limitations. We previously showed that derived changes in glycogen concentration were within experimental limits in excellent qualitative agreement with biochemical measurements. Given the fact that only the ^{13}C-labeled part of the glycogen molecule is measured, we sought to develop a method allowing absolute concentration measurement using NMR. The rationale for this method was to achieve near steady-state enrichment using long-term ^{13}C administration, to measure this enrichment *in vivo* which can then be matched by subsequent glucose infusion, thereby minimizing turnover as potential confound.
Materials and Methods: Male Sprague-Dawley rats (n=6) were fasted overnight with free access to water and then fed *ad libitum* for 48 h with a 50% enriched Glc solution (10% w/v) only. Then they were anesthetized using isoflurane, intubated, ventilated (for 1 h 45 min), placed into the magnet (9.4T) and indirect measurement of ^{13}C-NAA enrichment began. To minimize postmortem degradation, rats were sacrificed using a focused microwave fixation device, brains dissected and assayed for tissue glycogen and Glc concentrations using biochemical measurements. By labeling the Glc molecule in its first C position with ^{13}C, we expected the enrichment of acetyl–CoA (NAA's precursor) to be half of the ^{13}C -Glc enrichment. The method consisted of *in vivo* measurement of ^{13}C enrichment in NAA using ^{1}H spectroscopy. ^{13}C enrichment of NAA and Glc (as well as digested glycogen) was also evaluated *in vitro* by high field spectroscopy (14.1T, 600 MHz). Rats that did not drink a significant amount of ^{13}C-Glc (n=1), resulting in ^{13}C-NAA enrichment <5%, were excluded from the analysis.
Results: The biochemical measurements of brain glycogen (ave±SEM, 4.8±0.1) and brain Glc (3.8±0.3) were in excellent agreement with our previous results. In vitro NMR measurements of ^{13}C-labeled-NAA on brain extracts was within experimental error identical with the concomitant *in vivo* NMR measurements (t-test, P>0.05). The average ^{13}C-glycogen enrichment was 24±1% and NAA was approximately half of that of glycogen (Glycogen ^{13}C-enrichment/NAA ^{13}C-enrichment: 2.2±0.2).
Conclusion: The study demonstrates that after extended ^{13}C-labeled-Glc administration, glycogen is labeled, that this degree of labeling can be inferred from the *in vivo* enrichment of NAA. Therefore, we conclude that studies where the enrichment of the infusate nearly matches that of glycogen are feasible.
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0543

ER stress in the hypothalamus causes central leptin and insulin resistance in rodents

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Background and Aims: Recent study has demonstrated that endoplasmic reticulum (ER) stress is increased in the liver of obese animals and causes insulin resistance by inhibiting insulin signaling pathway. In the present study, we investigated whether ER stress may also involves a central resistance to insulin and leptin, major appetite regulating hormones, in the hypothalamus.
Materials and Methods: We measured ER stress markers (GRP78, CHOP) and JNK phosphorylation in the hypothalamus of high fat diet (60% fat, 7 weeks) induced obese mice. ER stress in the hypothalamic neurons wasinduced by intra-cerebroventricular (ICV) or intra-hypothalamic (arcuate nucleus) administration of thapsigargin or tunicamycin. We monitored food intake and body weight following ICV administration of insulin and leptin with or without administration of ER stress inducer.
Results: In the hypothalamus of high fat diet-fed mice, ER stress marker (GRP78, CHOP) and phospho-JNK expression was increased compared to low fat diet-fed controls. ICV administration of thapsigargin and tunicamycin prior to ICV leptin and insulin completely inhibited leptin and insulin-induced anorexia and weight loss. Bilateral injection of thapsigargin or tunicamycin into the hypothalamic arcuate nucleus also caused a similar inhibition on leptin and insulin-induced anorexia, suggesting that these effects are mediated via the hypothalamus.
Conclusion: The levels of ER stress markers are increased in the hypothalamus of obese animals. Induction of ER stress in the hypothalamus inhibits the effects of leptin and insulin and on food intake and body weight. These data may suggest

that increased ER stress in the hypothalamus of obese animals may cause central leptin and insulin resistance.
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Alzheimer-like tau phosphorylation induced by dysregulated insulin signal transduction and impaired glucose metabolism in type 1 diabetes mellitus

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Background and Aims: Abnormal hyperphosphorylation of tau plays a critical role in the pathogenesis of Alzheimer disease (AD) and diabetes is a known risk factor of AD. We therefore studied the roles of dysregulation of insulin signaling and glucose metabolism in hyperphosphorylation of tau in type 1 diabetic rats.
Materials and Methods: Wistar rats were randomized into 3 groups, pancreatectomy (PX), type 1 diabetes mellitus (T1DM) and control (CTL) group. The model of PX group characterized by low plasma insulin was made by partial (90%) pancreatectomy, and the model of T1DM group was made by single injection of STZ. The plasma insulin level was measured by RIA method, and the plasma glucose by glucose-oxidase method. Total tau level, the phosphorylation level of tau at individual phosphorylation sites (Ser199, Thr212, Ser214, Ser396 and Ser422) and glucose transport 3 (GLUT3) on neuronal membrane fractions were analyzed by Western blots. The activity of glycogen synthase kinase 3β (GSK-3 β), a key component of insulin signal transudation pathway and a known tau kinase, in the hippocampus of rats was determined by usingγ^{32}-P-ATP and the specific peptide substrate.
Results: We found that the plasma insul level was remarkedly decreased in T1DM and PX group as compared to CTL group, and plasma glucose level in T1DM was increased to ~3 times of the CTL, but that in PX group was only marginally increased. Neither pancreatectomy nor STZ injection changed the total level of tau protein in the hippocami of rats. However, Tau was found to be hyperphosphorylated at several AD-related phosphorylation sites (Ser199,Thr212, Ser214, Ser396 and Ser422) in T1DM group. In PX group, Ser199,Thr212 and Ser396 of tau protein were hyperphosphorylated, but no significant changes at Ser214 and Ser422. The activity of GSK-3β was found to be increased dramatically in the hippocampi of rats in both PX and T1DM groups. The level of GLUT3 was also found to be decreased in both PX and T1DM groups, with a more dramatic decrease in T1DM group.
Conclusion: These findings suggest that impaired insulin signaling could facilitate hyperphosphorylation of tau via both upregulation of GSK-3β and decreased intracellular glucose metabolism.

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Mitochondria

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Decreased mitochondrial respiration impairs insulin action in cultured human skeletal muscle cells

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Background and Aims: Skeletal muscle insulin resistance is a characteristic feature of type 2 diabetes. Insulin resistant type 2 diabetic patients have been shown to have impaired skeletal muscle mitochondrial respiratory function. A key question is whether the impaired mitochondrial function contributes directly to the decreased insulin action.
Materials and Methods: Human skeletal muscle cell cultures were used to develop a model of impaired cellular respiratory function to examine the effects upon insulin action. Human skeletal muscle cells were incubated with the mitochondrial inhibitor sodium azide, an inhibitor of complex IV of the mitochondrial respiratory chain. Cellular respiration was measured polarographically. By titrating the dose of the mitochondrial inhibitor, we aimed to mimic the decrease in skeletal muscle mitochondrial oxidation observed in the insulin resistant patients. Skeletal muscle cell cultures from 5 healthy subjects were incubated with increasing concentrations of sodium azide for 24 h before examining the effects of this inhibitor on respiration and glucose uptake.
Results: Incubation of human skeletal muscle cells with 50 μM and 75 μM sodium azide resulted in a 48±8.3% and 56±3.2% decrease in respiration compared to untreated cells. After incubation with 50 μM and 75 μM sodium azide, basal rates of glucose uptake were increased 2.1 and 2.7 fold, (both $p<0.0001$) respectively, compared to the untreated cells. In response to 100 nM insulin, glucose uptake was impaired in cells treated with sodium azide (n=6 for each culture). In untreated cells, the fold increase over basal in response to insulin was 1.49±0.05 (mean±SEM: vs basal $p=0.0003$). This was significantly decreased in cells treated with 50 μM azide (1.14±0.04 fold increase: vs

untreated p<0.0001) and 75 μM azide (1.25±0.02 fold increase: vs untreated p=0.0001). To investigate the underlying cause of the increased basal glucose uptake, Western blotting was performed using a phospho-AMPK(Thr^{172}) antibody. It was found that phosphorylation, and thus activation, of AMPK was substantially increased under conditions of impaired cellular respiration.

Conclusion: In conclusion, suppression of cellular respiration to levels comparable to those reported in insulin resistant subjects results in decreased responsiveness of glucose uptake to insulin in cultured human muscle cells. This decreased insulin action was accompanied by increased rates of basal glucose uptake and increased phosphorylation of AMPK.

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0546

Muscle fatty acid oxidative capacity and PGC-1α expression are upregulated in mouse models of insulin resistance and type 2 diabetes

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Background and Aims: Decreased muscle mitochondrial number as well as a reduced capacity for fatty acid oxidation by muscle mitochondria, have been suggested as key factors in the development of obesity, insulin resistance and type 2 diabetes. It is unclear however, whether these defects are due to genetic factors or are acquired (e.g. through dietary lipid excess or physical inactivity). In the current study we have investigated parameters of mitochondrial function and lipid metabolism in skeletal muscle of high-fat fed C57Bl/6J mice and compared the results to those obtained from a genetic model of type 2 diabetes, the *db/db* mouse.

Material and Methods: C57Bl/6J mice were fed a high-fat diet (Fat 45%, Protein 20%, CHO 35%) for either 5 wk or 20 wk and an ipGTT (2 g/kg) was conducted to confirm glucose intolerance. The activity of key enzymes involved in fat metabolism (citrate synthase (CS), β-hydroxyacyl-CoA dehydrogenase (βHAD) and medium chain acyl-CoA dehydrogenase (MCAD)), as well as the ^{14}C-palmitate oxidation rate were assessed in skeletal muscle homogenates. Immunoblotting was used to determine the expression levels of proteins involved in mitochondrial biogenesis and fatty acid metabolism, including PGC-1α, UCP3 and subunits of mitochondrial respiratory chain complexes.

Results: There was a highly significant (P<0.001) increase in epididymal fat mass as a result of the high-fat diet after both 5 wk (150%) and 20 wk (350%), with a significant (P<0.05) impairment in glucose tolerance also observed at both timepoints. A significant increase (P<0.01) in the activity of CS (25–60%), βHAD (30–70%) and MCAD (60–120%) was observed in the fat–fed animals compared with chow–fed controls. In muscle from *db/db* mice there was also a significant (P<0.01) elevation in the activity of CS (25%), βHAD (25%) and MCAD (55%) compared with lean controls (*db/+*). Fat–feeding substantially altered the expression of proteins involved in mitochondrial fatty acid metabolism with a significant (P<0.05) increase observed for PGC-1α (100–250%), UCP3 (80–240%), Complex III (65–110%) and Complex IV (50–140%). These results were paralleled by those observed in *db/db* mice, where there was a significant (P<0.05) elevation in the protein expression of PGC-1α (45%), UCP3 (120%) and Complex III (35%) compared with *db/+* controls. To gain a direct measure of fatty acid oxidative capacity we assessed palmitate oxidation rate in tissue homogenates from a separate group of fat–fed animals (20 wk), and found a marked improvement in fatty acid oxidative capacity compared with chow–fed controls (3.60±0.14 vs 2.34±0.16 μmoles palmitate/g/hr, P<0.001).

Conclusions: In summary, our results show that in both high-fat fed mice and *db/db* mice there is an improved fatty acid oxidative capacity and an upregulated expression of proteins related to mitochondrial function. We therefore conclude that defects in muscle mitochondrial function are not responsible for the obesity, insulin resistance and type 2 diabetes observed in these mouse models.

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Expression of mitochondrial genes related to energy homoeostasis are dysregulated in obesity and type 2 diabetes

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Background and Aims: The integrity of mitochondria in adipose tissue in obesity and type 2 diabetes (T2DM) has recently been investigated in animal models but to date limited data is available in humans. The primary defect(s) causing bioenergetic dysfunction may reside in a non-

bioenergetic pathways such as signaling between mitochondria and nucleus or in overall mitochondrial biogenesis or degradation pathways impacted by inflammation. Therefore we investigated a number of mitochondrial genes including PPARγ co-activator 1 (PGClα, mitochondrial biogenensis), UCP-2 (fatty acid oxidation), COX4 (flux-generating reactions in the electron transfer chain), fatty acid synthase (FAS, catalysing the synthesis of long chain saturated fatty acids) and SIRT3 (implicated in inhibition of PGC1α).

Materials and Methods: Utilising real time PCR and Western blot for SIRT3, we examined the effect of obesity and T2DM status, as well as the relative influence of adipose tissue (AT) depots (abdominal subcutaneous (Abd Sc) versus omental (Om) AT). For this study, we collected a lean non-diabetic cohort (BMI: 22.2±(Mean±SD)2.1 kg/m^2, age: 39.8±(Mean±SD)8.7 yrs, n=12), obese non-diabetic cohort (BMI:30.1±2.81 kg/m^2, age: 47.3±11.5 yrs, n=9) and T2DM cohort (BMI: 59.9±7.5 kg/m^2, age: 37.15±7.8 yrs, n=7) of human adipose tissue (AT).

Results: Our findings determined that in Abd Sc AT increasing adiposity significantly reduced FAS ($p=0.013$), COX4 ($p=0.0003$) and UCP2 ($p<0.001$) mRNA, whilst PGC1α ($p=0.003$) mRNA was increased. In addition, in Abd Sc AT from T2DM subjects there was a reduction in FAS, COX4, UCP-2 mRNA ($p<0.05$); whilst PGC1α mRNA ($p=0.0004$) was again increased. In Om fat, FAS, PGC1α, COX4 and UCP2 mRNA were all reduced ($p<0.001$). Depot specific differences were also observed. SIRT 3 protein expression highlighted that there was no significant effect with adiposity in either depot, with a reduction in T2DM Abd Sc AT protein expression (Abd Sc Lean vs T2DM, $p=0.002$).

Conclusion: In summary, these studies highlight that in Abd Sc AT, whilst PGC1α mRNA (related to biogenesis of mitochondria) continues to increase in obesity, the genes related to energy metabolism appears to reduce with a similar pattern observed in Abd Sc AT from T2DM subjects. In Om AT, PGC1α mRNA also reduces along with genes related to mitochondrial metabolism. Finally, SIRT 3 implicated in inhibiting PGC1α in rodent models is also reduced in AT from T2DM subjects. In conclusion, these studies suggest a role for mitochondria in human adipose tissue appears dysregulated in obesity and diabetes. The causes of mitochondrial dysregulation are unclear, however candidate factors may include oxidant stress as well as lipotoxicity.

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TNFα impairs mitochondrial metabolism in 3T3-L1 adipocytes

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Background and Aims: The adipose tissue is now recognized as an endocrine/paracrine organ which can influence whole body metabolism not only through the release of non-esterified free fatty acids but also via many adipokines such as tumor necrosis factor α (TNFα). TNFα is a proinflammatory cytokine, which has been implicated as a contributing cause of insulin resistance. Although the effects of TNFα are well known, the mechanisms by which TNFα impairs insulin action are not well understood. A number of recent studies support the concept that insulin resistance is linked to mitochondrial dysfunction. The aim of this study was to investigate whether TNFα may affect pathways involved in mitochondrial biogenesis and fatty acid oxidation in 3T3-L1 adipocytes.

Materials and Methods: Differentiated 3T3-L1 adipocytes were treated with TNFα (3 ng/ml, 72 hrs) and in the final 24 hrs of that incubation, cells were also treated with troglitazone (TGZ; 10 μM), and aspirin (ASA; 5 mM). Insulin sensitivity was determined by 2-deoxyglucose uptake and phosphorylation of key proteins in insulin signaling pathway. Following treatments, RNA was extracted and hybridised to custom-made cDNA microarrays containing ~12,000 elements. Real-Time PCR of mitochondrial-specific target genes was also performed to confirm microarray data.

Results: Insulin-stimulated glucose uptake was inhibited by 30–40% ($p\leq0.0002$ compared to vehicle, $n\geq10$) when 3T3-L1 adipocytes were exposed to TNFα for 72 hrs. Treatment with TGZ and/or ASA alleviated TNFα-induced inhibition of insulin-stimulated glucose uptake ($p\leq0.007$ compared to TNFα alone, $n\geq6$). Microarray analysis of 3T3-L1 adipocytes treated with TNFα±TGZ and ASA (n=20 replicates per treatment) identified significant changes in the expression of several genes encoding key mitochondrial proteins such as citrate synthase (CS), CPT-1, PGC1α. CPT1, PGC1α and CS gene expression were all decreased following TNFα treatment and restored to basal levels with TGZ/ASA treatment. Real time PCR analysis confirmed the microarray results and showed that PGC1α mRNA levels were markedly decreased following TNFα treatment ($p<10^{-5}$). Treatment with TGZ/ASA was reversed the suppressed PGC1α gene expression following TNFα treatment ($p<10^{-4}$).

Conclusion: Our results showed that TNFα not only impairs insulin-stimulated glucose uptake but also genes encoding key mitochondrial proteins, particularly those involved in the regulation of fatty acid oxidation (PGC1α, CPT1, CS, PPARα) but not genes involved in mitochondrial biogenesis (mtTFA, NRF1, PPARγ).

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Reduced expression of nuclear-encoded genes involved in mitochondrial oxidative metabolism in skeletal muscle of obese women with polycystic ovary syndrome (PCOS)

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Background and Aims: Recently, abnormalities in mitochondrial oxidative phosphorylation (OXPHOS) have been implicated in the pathogenesis of skeletal muscle insulin resistance in type 2 diabetes. In the present study, we hypothesized that decreased expression of OXPHOS genes could be of similar importance for insulin resistance in the polycystic ovary syndrome (PCOS).
Materials and Methods: Using the HG-U133 Plus 2.0 expression array from Affymetrix, we analyzed gene expression in skeletal muscle from obese women with PCOS (n=16) and age- and body mass index-matched control women (n=13) metabolically characterized by euglycemic-hyperinsulinemic clamp and indirect calorimetry. First, Welch's two sample t-test was applied to examine the significance of OXPHOS genes separately. The overall significance of the OXPHOS genes was assessed by calculating a sum statistic and conducting a permutation test. Subsequently, we performed biological pathway analysis using Gene Set Enrichment Analysis (GSEA) and Gene Microarray Pathway Profiler (GenMAPP).
Results: Women with PCOS were characterized by fasting hyperinsulinemia and impaired insulin-stimulated glucose disposal – caused by reduced glucose oxidation and storage – as well as impaired suppression of lipid oxidation (all $P<0.01$). These abnormalities were associated with a significant decrease in the selected subset of nuclear-encoded genes involved in mitochondrial OXPHOS ($P=0.025$). GSEA and GenMAPP both revealed the same set of genes involved in OXPHOS, which was also the most downregulated biological pathway ($P<0.01$). These findings are currently being validated by quantitative real-time PCR and immunoblot analysis.
Conclusion: Our results, for the first time, provide evidence for an association between insulin resistance and impaired mitochondrial oxidative metabolism in skeletal muscle in women with PCOS. This may contribute to the increased risk of type 2 diabetes observed in these women.
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0550

The autonomic nervous system could influence the skeletal muscle metabolic activity by regulating the expression of PGC-1alpha and Mfn-2

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Background and Aims: Imbalanced autonomic nervous system (ANS), reduced glucose uptake as well as impaired mitochondrial function at the level of skeletal muscle are part of the metabolic alterations observed in obesity. Peroxisome proliferator-activated receptor gamma coactivator (PGC-1alpha) is a nuclear transcriptional co-activator that regulates mitochondrial biogenesis and adaptive thermogenesis. Mitofusin-2 (Mfn-2) is an other key factor involved in the formation and maintenance of the mitochondrial network. Decreased expression of PCG-1alpha and Mfn2 has been suggested to participate in the pathogenesis of insulin resistance in obese and/or diabetic patients. In turn, insulin resistance could contribute to the development of ANS dysfunction. We therefore designed a study aimed at investigating possible links between the mRNA expression of PGC-1alpha and Mfn2, insulin resistance in muscle and the cardiac autonomic balance.
Materials and Methods: 23 morbidly obese women (BMI: 46±4 kg/m^2, age: 37±9 y) exempt from diabetes were investigated. Cardiac autonomic balance was investigated by power spectral analysis of Heart Rate Variability (HRV) during a 30-minute period of continuous ECG record; insulin sensitivity was assessed by an euglycaemic-hyperinsulinaemic clamp; RT-PCR was used to measure mRNA expression of PGC-1alpha and Mfn-2 in skeletal muscle biopsies.
Results: Glucose uptake was 3,5±0.4 mg/kg LBM/min, indicating a reduced insulin sensitivity. The HRV parameters were: Low Frequency=53±4 NU, High frequency=35±4 NU, Low-to-High ratio=2.0±0.3, suggesting a sympathetic prevalence at the heart level. PGC-1alpha value was 0.51 AU and Mfn-2 0.37 AU. A significant, positive correlation linked the mRNA expression of PGC-1alpha and the cardiac autonomic parameters mostly influenced by the sympathetic activity: Low Frequency ($p=0.003$), Low-to-High Frequency ratio ($p=0.001$). Similar results were found with the mRNA expression of Mfn-2. A strong positive relationship linked the mRNA expression of PGC-1alpha and Mfn-2 ($p<0.0001$). However, glucose uptake did not show any significant relationship with the

cardiac autonomic parameters, nor with the expression of PGC-1alpha and Mfn-2.
Conclusion: In morbidly obese subjects, an elevated mRNA expression of PGC-1alpha and Mfn-2 in skeletal muscle corresponds to a high sympathetic tone at the cardiac level. The knowledge that genes participating in carbohydrate and lipid metabolism are regulated by epinephrine in human skeletal muscle support the hypothesis that ANS activity could be concomitantly and in parallel regulated in the cardiac and muscle district. Furthermore, the ANS could influence the skeletal muscle metabolic activity by regulating the expression of PGC-1alpha and Mfn-2.
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PS 34

Effects of glitazones on insulin sensitivity

0551

Long-term pioglitazone treatment prevent age-related impairment of tissues insulin sensitivity and adiponectin secretion in sucrose fed SHR rats

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Background and Aims: Thiazolidinediones (TZDs) are peroxisome proliferator activated receptor-gamma (PPAR-γ) agonists used for treatment of type 2 diabetes. TZDs improve glycemic control by reducing insulin resistance in target tissues, however, mechanisms of their action are not fully understood. The aim of the current study was to analyze long-term effects of pioglitazone on insulin and adiponectin secretion, muscle lipid accumulation a peripheral tissue insulin sensitivity in an animal model of the metabolic syndrome, the spontaneously hypertensive rats.
Materials and Methods: Groups of adult male rats were fed a high carbohydrate diet (70 cal% sucrose) from the age of 4 to 8 months without or with pioglitazone (300 mg/kg). The insulin sensitivity of adipose tissue and skeletal muscle tissues was measured by incorporation of ^{14}C-U glucose into lipids and glycogen, respectively. Serum insulin concentrations were determined using Mercodia Rat Insulin ELISA kit (Mercodia AB, Uppsala, Sweden) and adiponectin concentrations by using Rat Adiponektin ELISA kit (B-Bridge International, CA, USA).
Results: Body weight gains were similar in control and experimental groups. Pioglitazone treatment markedly reduced serum concentrations of triglycerides (0.73±0.05 vs 0.52±0.01 mmol/l, P<0.005), fasting serum glucose (5.1±0.2 vs 4.6±0.1 mmol/l, P<0.05) and prevented age-related development of hyperinsulinemia (0.454±0.040 vs 0.135±0.018, nmol/l, P<0.0005). In addition, pioglitazone treated rats exhibited significantly increased concentrations of serum adiponectin (5.8±0.6 vs 8.9±0.1 μg/ml, P<0.005). Compared to controls, pioglitazone treatment was associated with redistribution of fat stores, with significantly increased epididymal fat pad weight (1.41±0.057 vs 1.65±0.076 g, P<0.05) and muscle triglycerides (2.25± 0.12 vs 3.83±0.33 μmol/g, P<0.005) and with a parallel amelioration of hepatic steatosis (7.75±0.31 vs 6.21± 0.16 μmol/g, P<0.005). Remodeled adipose tissue contained higher percentage of protein (1.17±0.03 vs 1.48±0.06%, P<0.005) and was metabolically more active, exhibiting significantly increased basal and adrenaline-stimulated lipolysis (P<0.005) and elevated sensitivity to insulin action as estimated by insulin stimulated ^{14}C-glucose incorporation into lipids (967±74 vs 679±63 nmol glucose/g/2 h, P<0.01). Surprisingly, pioglitazone treated rats exhibited increased sensitivity of muscle tissue to insulin stimulated glucose incorporation into glycogen (774±58 vs 1288±63 nmol glucose/g/2 h, P<0.0001, P<0.005) despite increased muscle triglyceride accumulation.
Conclusion: Our results provide evidence for long-term beneficial therapeutical effects of pioglitazone due to its effects on adipose tissue remodeling and on modulation of adiponectin secretion. Amelioration of insulin resistance in skeletal muscles was independent on reduction of muscle triglyceride storage. We conclude that pioglitazone due to lower insulin secretory demand might preserve beta-cell function and slow down age-related progression of insulin resistance.
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0552

First-line therapy with fixed-dose combination of rosiglitazone plus glimepiride improves insulin sensitivity and reduces cardiovascular risk markers in patients with type 2 diabetes

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Background and Aims: The long-term complications observed in patients with type 2 diabetes (T2D) are caused by a number of conditions, including insulin resistance and vascular inflammation, as well as poor glycaemic control. It has been suggested that early and aggressive treatment with an effective antihyperglycaemic therapy that also has beneficial effects on insulin resistance and vascular inflammation may have long-term benefits. The aim of this study was to evaluate the effect of a fixed-dose combination (FDC) of rosiglitazone (RSG) and glimepiride (GLIM), in comparison with RSG and GLIM monotherapies, on insulin resistance, β-cell function and biomarkers of atherosclerotic cardiovascular risk in drug-naive individuals with T2D.
Materials and Methods: Patients were globally randomized to receive double-blind treatment with either GLIM monotherapy [1–4 mg once daily (OD)], RSG monotherapy (4–8 mg OD), RSG/GLIM FDC A (4 mg/1–4 mg OD) or RSG/GLIM FDC B (4–8 mg/1–4 mg OD) for 28 weeks. Treatment was started on the lowest dose and uptitrated at 4-week intervals to achieve a mean daily glucose level of <6.1 mmol/l. Insulin sensitivity [as estimated by homeostasis model assessment (HOMA)-S], β-cell function (as estimated by HOMA-B) and the levels of cardiovascular biomarkers were determined at the end of the study.
Results: A total of 901 patients were involved in the study. Increased adiponectin levels correlated with improvements in insulin sensitivity (HOMA-S) with both FDC regimens and with RSG monotherapy but not with GLIM monotherapy. Treatment with either FDC regimen resulted in greater increases in β-cell function (HOMA-B) than did either GLIM or RSG monotherapy. There were greater reductions in the level of C-reactive protein (CRP) and the activity of plasminogen activator inhibitor-1 (PAI-1) following treatment with either FDC or with RSG compared with GLIM monotherapy. There was a smaller increase in LDL cholesterol (LDL-C) in both FDC treatment groups compared with RSG monotherapy and the level of HDL cholesterol (HDL-C) was increased following treatment with either FDC regimen or with RSG monotherapy.

% Δ **from** **Baseline**	GLIM n=221	RSG n=227	FDC A n=221	FDC B n=214
Adiponectin	−5.3	128.5	65.7*,†	116.46*
HOMA–S	−3.2	36.3	21.9*,‡	23.0*,§
HOMA–B	58.7	46.1	73.4 °,*	105.8*,†
CRP	−7.9	−39.8	−43.6*,†	−50.7*,†
PAI–1	−4.5	−22.5	−20.1	−19.1¥
LDL–C	−2.1	9.4	2.6¥,§	4.2¥
HDL–C	−1.2	5.2	3.8¥	2.8

*p<0.0001 vs. GLIM; †p<0.0001 vs. RSG; ‡p<0.01 vs. RSG; §p<0.05 vs. RSG; ¥p<0.05 vs.GLIM; °p<0.001 vs. GLIM;

Conclusion: These results suggest that early aggressive treatment with RSG/GLIM FDC may reduce vascular inflammation and enhance insulin sensitivity in drug-naive patients with T2D.

0553

Effects of pioglitazone on glucose metabolism and blood pressure in type 2 diabetic patients in relation to physical activity – The IRIS III Study

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Background and Aims: Improvement of insulin sensitivity by physical activation or pharmaceutical intervention with PPARγ-agonists in type 2 diabetic patients was shown to improve glucose metabolism and to reduce several cardiovascular risk factors. There is only little information available about the interactions of physical activity and PPARγ stimulation on insulin resistance, glucose metabolism, and blood pressure in type 2 diabetic patients. The aim of our investigation was to explore the effect of pioglitazone treatment in type 2 diabetic patients according to different levels of physical activity.
Materials and Methods: One-thousand-nine-hundred-thirty patients with type 2 diabetes (1020 male, 910 female; age 63.1±10.4 years; duration of diabetes 6.1±5.0 years; mean±SD) were included in this observational trial and received treatment with 45 mg Pioglitazone. At baseline and after twenty weeks of treatment with Pioglitazone, fasting glucose, HbA1c, systolic and diastolic blood pressure, and the body mass index were documented. According to the frequency and extend of their physical activity, the patients were classified in three different groups with no physical activity, occasional physical activity and frequent physical activity.
Results: As shown in table 1, patients reporting occasional or frequent physical activity were presented with significantly lower fasting glucose, HbA1c, BMI, and blood pressure measurements at baseline compared with type 2 diabetic patients without physical activity. Despite the absolute reduction in fasting glucose, HbA1c and diastolic blood pressure was somewhat lower in the patient groups with occasional or frequent physical activity compared with those without physical activity (p<0.05, respectively), treatment with pioglitazone resulted in a significant improvement in fasting glucose, HbA1c, systolic and diastolic blood pressure in all three patient groups. No

Physical Activity	Without	Without	Without	Occasional	Occasional	Occasional	Frequent	Frequent	Frequent
n=	478	478	478	1136	1136	1136	316	316	316
	Baseline	20 weeks	delta	Baseline	20 weeks	delta	Baseline	20 weeks	delta
Fasting Glucose (mmol/L)	9.5±2.6	7.9±2.5 $	−1.6±2.9	9.1±2.6 §	8.8±2.3 $	−1.3±2.6	9.1±2.6 §	8.0±2.5 $	−1.0±2.8 §
HbA1c (%)	8.0±1.4	7.1±1.3 $	−0.9±1.3	7.7±1.3§	7.0±1.2 $	−0.7±1.2	7.8±1.2 §	7.0±1.1 $	−0.7±1.1
RR syst. (mmHg)	142±16	137±14 $	−4.45±14.4	140±15 §	136±14 $	−2.7±12.2 §	140±16	139±15	−1.5±14.4$
RR diast. (mmHg)	82.1±8.6	82.2±7.9	0.1±8.7	81.3±7.7 §	80.9±7.2	−0.4±7.4	80.9±8.9 §	81.5±7.2	0.8±8.3
BMI (kg/m^2)	31.7±6.0	31.8±6.4	−0.2±1.3	30.3±5.1 §	30.1±5.2	0.0±1.7	29.6±4.6 §	29.7±4.8	0.1±1.2 §

change in BMI could be observed during Pioglitazone treatment in any of the different physical activity groups.

Conclusion: As expected, in our observational trial type 2 diabetic patients without physical activity presented with worse glucose control and higher blood pressure measurements at baseline. Nevertheless, also in type 2 diabetic patients at a high physical activity level treatment with pioglitazone still evolves additional clinical effects on glucose control and blood pressure.

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0554

Metabolic effects of telmisartan and irbesartan in type 2 diabetic patients with metabolic syndrome treated with rosiglitazone: a double-blind, randomised clinical trial

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Background and Aims: Angiotensin II receptor blockers represent a class of effective and well tolerated orally active antihypertensive drugs, both in general population and diabetic patients. The aim of our study was to investigate the metabolic effect of telmisartan and irbesartan in subjects treated with rosiglitazone, a well-known insulin sensitizing drug, in order to discriminate eventual direct metabolic effects of the studied molecules.

Materials and Methods: Patients were enrolled, evaluated, and followed at 3 Italian Centres. We evaluated 188 type 2 diabetic patients with metabolic syndrome (94 males and 94 females; 49 males and 46 females, aged 56±5 with telmisartan; 45 males and 48 females, aged 55±4 with irbesartan). All were required to have been diagnosed as being diabetic for at least 6 months, and did not have glycemic control with diet and oral hypoglycemic agents, both to the maximum tolerated dose. All patients took a fixed dose of rosiglitazone, 4 mg/day. We administered telmisartan (40 mg/day) or irbesartan (150 mg/day) in a randomized, controlled, double-blind clinical study. We evaluated BMI, glycemic control (HbA_{1c}, FPG, FPI, and Homa-index), lipid profile (TC, LDL-C, HDL-C, and Tg), SBP and DBP, adiponectin, resistin, TNF-α, and leptin during 12 months of this treatment.

Results: No BMI change was observed after 6, and 12 months in both groups. Significant HbA_1c and FPG decrease was observed after 6 months in telmisartan group, and after 12 months in both groups. HbA_1c and FPG decrease was significantly lower after 12 months in telmisartan group. Significant FPI decrease was obtained at 12 months in both groups, and this decrease was significantly lower in telmisartan group. Significant Homa index decrease was obtained at 6, and 12 months in both groups, and Homa index lowering was significant in telmisartan group after 12 months. Significant SBP, DBP, TC, and LDL-C change was observed after 6, and 12 months in both groups. Adiponectin concentration was increased after 6 months in telmisartan group, and after 12 months in both groups and this difference was significant after 12 months in telmisartan group. Significant resistin decrease was obtained after 6 months, and 12 months in telmisartan group, whereas it was observed a significant change at 12 months in irbesartan group and this difference was significant after 12 months in telmisartan group. Significant TNF-α and leptin levels decrease was observed after 6 months in telmisartan group, and after 12 months in both groups.

Conclusion: In this study of patients with type 2 diabetes mellitus and metabolic syndrome, telmisartan seemed to improve glycemic and lipid control and metabolic parameters regarding metabolic syndrome, compared to irbesartan. These observed metabolic effects of different AT(1) receptor blockers could be relevant in the therapy choice for the correct management of patients affected by metabolic syndrome and diabetes.

0555

Effects of short-term metformin and rosiglitazone treatment on GLP-1, leptin and adiponectin levels in patients with type 2 diabetes mellitus

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Background and Aims: Type 2 diabetes mellitus (DM) is a chronic progressive disorder with an increasing prevalance. Decreased insulin secretion and peripheral insulin resistance are the two major pathogenetic factors in the disease. Disordered glucagon like peptide (GLP)-1 production have been shown in diabetic patients. GLP-1 is an incretin that increases insulin secretion after food intake and plays role in the regulation of plasma glucose levels. Increased leptin and decreased adiponectin levels have been reported in patients with insulin resistance. Metformin and rosiglitazone are used in the treatment of type 2 DM. These drugs act by improving insulin sensitivity.
The aim of this study was to evaluate the short-term effects of metformin and rosiglitazone on serum levels of GLP-1, leptin and adiponectin in type 2 diabetic patients.
Materials and Methods: Forty-one patients with type 2 DM were included to the study. Serum GLP-1, leptin, adiponectin, glucose and insulin levels were measured before and 30, 60, 90, 120 minutes after ingestion of a mixed meal at the initiation of the study. After basal evaluations patients were divided into three groups and either metformin (n=15, 1700 mg/day) or rosiglitazone (n=15, 4 mg/day) or placebo (n=11) was started. All above mentioned parameters were measured after 14 days of drug treatments.
Results: Insulin resistance which was calculated as HOMA-IR decreased significantly in both metformin and rosiglitazone groups (6.4±2.8 vs. 2.3±0.8 ; $p<0.01$ and 6.8±3.5 vs. 3.2±1.1; $p<0.01$; respectively). No changes were observed in GLP-1 levels in the groups. Basal and peak leptin levels decreased in both drug groups (38.5±16.6 vs. 35.0±14.1 ng/ml; $p<0.05$ and 36.3±14.8 vs. 33.3± 15.5 ng/ml; $p<0.05$ in metformin group; and 37.8±19.7 vs. 35.4±18.1 ng/ml; $p<0.05$ and 40.1±17.5 vs 35.3±15.6 ng/ml; $p<0.05$ in rosiglitazone group). Adiponectin levels increased significantly in rosiglitsone group (4.0±2.0 vs 5.6±1.3 ng/ml; $p<0.05$ and 4.6±1.8 vs 7.4±2.1 ng/ml; $p<0.05$ basal and peak levels, respectively). An increase in adiponectin levels was also observed in the metformin group but it was not statistically significant. HOMA-IR, leptin and adiponectin levels did not change in the placebo group.
Conclusion: In conclusion, both rosiglitazone and metformin decreased leptin levels and increased adiponectin levels by improving insulin resistance in this short-term study. No effect on GLP-1 levels could be found in the present study but more long-term longitudinal studies are needed to confirm this finding.

0556

Genome-wide investigation of genes regulated in different cell fractions of adipose tissue from insulin-resistant subjects by short-term treatment with pioglitazone

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Background and Aims: Insulin resistance is a major predictor for the development of type 2 diabetes. Thiazolidinediones (TZDs) are commonly used insulin sensitizing drugs that activate nuclear receptor PPARs, leading to adipogenesis and improvement in systemic insulin sensitivity. The genes regulated by TZD treatment may therefore potentially contribute to the development of insulin resistance and hence type 2 diabetes.
Materials and Methods: We treated five insulin-resistant first-degree relatives of type 2 diabetic patients (age 51.2+2.6 yrs, BMI 28.1+1.5 kg/m^2) with pioglitazone at 30 mg/day for three weeks, and investigated gene expression in isolated adipocytes and adipose tissue using Affymetrix U133 GeneChip® for human genome. The microarray measurements were verified by TaqMan real-time PCR. This study design is thought to provide two advantages; 1) the short-term treatment eliminates the potential confounding effects resulting from significant body weight gain that is often associated with long-term treatment, 2) the GeneChip® detection algorithm permits segregation of genes into those expressed in adipocytes, in non-adipocytes, or in the both cell fractions of adipose tissue.
Results: Body weight and BMI were not altered after the treatment. Fasting plasma glucose (5.1+0.2 vs. 4.7+0.2 mM, P=0.05) and insulin (12.9+1.2 vs.11.0+0.9 mU/L) were numerically decreased. Glucose disposal rate was increased from 6.9+0.5 before to 8.1+0.6 mg/kg LBM/min after the treatment ($P<0.01$). There were 325 transcripts up-regulated and 142 down-regulated in the adipocytes and 109 up-regulated and 90 down-regulated in the adipose tissue. Functional classification of these transcripts suggested a transcriptional activation of pathways essential for adipocyte energy storage, prominently fatty acid esterification and glucose metabolism (Table). Many genes enrolling in cell division or differentiation and in tissue remodeling were also found to be expressed differentially after the treament. Approximately 30 genes were induced (absent before the treatment and present after) or suppressed (present before the treatment and absent after) in either adipocytes or non-adipocytes by the treatment. In addition,

37 genes, mostly stress-responding genes, were induced (absent in adipose tissue but present in isolated adipocytes) by the standard adipocyte isolation procedure.

Conclusion: Short-term treatment with pioglitazone improves systemic insulin sensitivity by increasing energy partitioning to the adipose tissue before significant adipogenesis occurs (estimated from the number and scope of adipogenic gene induction and body weight gain). Further study of the genes responding to TZDs at the early stage of treatment before significant body weight gain may enable the identification of gene candidates that predispose to insulin resistance and/or type 2 diabetes.

Table. Selected genes up-regulated in adipocytes by short-term treatment with pioglitazone

Genes	Fold Increase
Acetyl–CoA carboxylase	1.7
Acetyl–CoA synthetase	1.4
Acyl–CoA synthetase long-chain	1.5
Acetyl–CoA acyltransferase	1.3
Acyglycerol-3-phosphate O-acyltransferase	2.5
Diacylglycerol O-acyltransferase	1.2
Phosphoenolpyruvate carboxykinase	2.0
Hexokinase	1.3
Pyruvate carboxylase	1.4
Pyruvate dehydrogenase	1.4
Fatty acid binding protein 5	1.8

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0557

Depot-specific regulation of perilipin by rosiglitazone in a diabetic animal model

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Background and Aims: Rosiglitazone, an insulin sensitizer of thiazolidinedione (TZD) class, is known as potent agonist for the peroxisome proliferator-activated receptor-γ (PPARγ) and promote differentiation of pre-adipocytes into adipocytes, resulting in lipid storage coupled with reduced release of free fatty acids into the circulation. Many studies reported that TZDs increase subcutaneous adiposity but have no effect on the visceral fat mass. Perilipin, a family of phosphoproteins located around lipid droplets in adipocytes, is essential for the enlargement of lipid droplets and lipolytic reaction by hormone-sensitivie lipase. Recently, a functional PPAR-responsive element (PPRE) was identified within the murine perilipin gene. We hypothesized that the depot-specific regulation of perilipin by rosiglitazone may be associated with the fat redistribution and insulin sensitizing effects of rosiglitazone.

Materials and Methods: We examined the change of adiposity, triglyceride contents in liver and muscle, morphological evaluation of pancreas after 6 weeks of treatment with placebo or rosiglitazone in OLETF rats, a type 2 diabetic animal model. Perilipin mRNA and protein expression were assessed in subcutaneous and epididymal fat using semi-quantitative RT-PCR and Western blotting.

Results: Rosiglitazone increased subcutaneous adiposity and decreased triglyceride contents of liver and muscle. Rosiglitazone decreased plasma free fatty acids (2107.3±507.4 in placebo group vs. 823.9±148.3 in rosiglitazone group, $p<0.05$) and triglyceride concentration (164.0±37.9 in placebo group vs. 56.8±14.7 in rosiglitazone group, $p<0.05$) and improved insulin resistance. The islets of placebo-treated rats showed hypertrophy and destruction, while the islets of rosiglitazone-treated rats showed hypertrophy but architecture intact. Perilipin mRNA and protein expression increased in subcutaneous fat, but did not change in visceral fat by rosiglitazone. In 3T3-L1 cell, rosiglitazone pretreatment decreased lipolysis and increased perilipin protein.

Conclusion: From the results, the increased perilipin expression in subcutaneous fat after rosiglitazone treatment is likely to be a mediator of reduced lipolysis, resulting in lipid storage in subcutaneous fat, the fat redistribution and insulin sensitizing effect.

PS 35

Pathophysiology, metabolism

0558

The influence of PPAR-α agonist treatment on insulin sensitivity in patients with type 2 diabetes: the role of resistin and adiponectin

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Background and Aims: Treatment with PPAR-α agonists – fibrates markedly improves insulin sensitivity in different rodent models of obesity and diabetes. Its influence on insulin sensitivity in humans is less clear. The aim of this study was to evaluate the influence of 3-months administration of PPAR-α agonist fenofibrate (200 mg/day) on

insulin sensitivity in obese patients with type 2 diabetes. In addition, possible involvement of the changes of circulating levels of adipose tissue-derived hormones adiponectin and resistin were also studied.

Materials and Methods: Basic anthropometric and biochemical parameters, insulin sensitivity and serum concentrations of adiponectin and resistin were measured in 12 obese female patients with type 2 diabetes (DM) and 12 lean healthy control women before and after 3 months of treatment with fenofibrate (200 mg/day). Insulin sensitivity was measured by isoglycaemic–hyperinsulinaemic clamp. Serum concentrations of resistin and adiponectin were assessed by commercial ELISA kits (BioVendor, CZ and Linco Research, USA, respectively).

Results: Body mass index, blood glucose, serum triglycerides, glycated hemoglobin and atherogenic index were significantly higher in DM relative to control group. In contrast, serum adiponectin and HDL cholesterol were lower in DM than in controls, while no difference between the two groups was found in serum resistin levels. Fenofibrate treatment significantly decreased serum triglycerides (2.07±0.30 vs. 3.12±0.50 mg/dl, $p<0.01$), but did not affect any other parameters including BMI and diabetes compensation. 3-months of fenofibrate treatment did not affect either adiponectin or resistin levels. Insulin sensitivity measured by isoglycaemic–hyperinsulinaemic clamp was markedly decreased in DM relative to control group. 3-months of fenofibrate treatment significantly improved glucose disposal rate (4.20±0.70 vs. 3.43±0.68 $\mu mol.kg^{-1}.min^{-1}$, $p<0.05$) but did not significantly affect either metabolic clearance rate of glucose or insulin sensitivity index in patients with type 2 diabetes mellitus.

Conclusion: In contrast to experimental studies, 3-months treatment of obese patients with type 2 diabetes mellitus with PPAR-α agonist fenofibrate had only minor effects on insulin sensitivity. Metabolic effects of PPAR-α agonists were not mediated by the changes of circulating levels of resistin or adiponectin.

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0559

Pharmacological PPARα activation induces substantial alterations in amino acid metabolism in fat fed, insulin resistant rats

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Background and Aims: Effects of PPARα agonists have been characterized largely in terms of their influence on lipids and glucose metabolism, but little has been reported about amino acid metabolism. The aim of this study was to provide further insight into effects of the PPARα agonist WY 14,643 with particular emphasis on treatment related changes in amino acid metabolism in fat–fed rats, a model of dyslipidaemia and insulin resistance.

Materials and Methods: WY 14,643 (30 μmol/kg/d for 4 wk) was administered to Sprague–Dawley rats fed a saturated fat diet. An initial metabonomics study was conducted, involving ^{1}H NMR analysis of plasma and urine samples. In addition, plasma amino acid levels were measured using HPLC and hepatic gene expression was assessed by measurement of mRNA levels.

Results: The high fat diet elevated plasma levels of insulin and triglycerides and WY 14,643 treatment ameliorated this hyperinsulinaemia and hypertriglyceridaemia. In addition, treatment decreased body weight gain, without altering cumulative food intake and increased liver mass. WY 14,643 increased plasma levels of 12 out of 22 amino acids compared to fat–fed controls, most noticeably for serine (+66%), glycine (+82%) and threonine (+78%), while arginine was significantly decreased. There was no alteration in branched chain amino acid levels. Compared with the fat fed control animals, WY 14,643 treated animals had raised plasma urea and ammonia levels. The methyl carriers, N-methylnicotinamide and dimethylglycine, were also elevated in urine of treated animals. WY 14,643 increased hepatic expression of a number of key genes involved in amino acid and fatty acid catabolism, including genes involved in ketone body formation.

Conclusion: The results show that in rodents pharmacological PPARα activation increases whole body amino acid mobilisation and catabolism thus extending the role of this transcription factor beyond the control of lipid and carbohydrate metabolism.

0560

Effects of physiological vs non-physiological restoration of postprandial insulin secretion on glucose and lipid metabolism in type 2 diabetes

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Background and Aims: The early increase in insulin secretion has a critical role in the regulation of postprandial glucose and lipid responses. Early in the course of type 2 diabetes (T2D), the increase in insulin secretion after a meal is delayed. However, the impact of this defect on postprandial glucose and lipid metabolism is questionable. This was investigated in T2D patients by giving nateglinide or glibenclamide (a rapid-acting and slower-acting insulin secretagogue respectively) prior to a meal. To avoid the confounding effects of chronic amelioration of hyperglycaemia on the parameters investigated, the experiments were performed after a single administration of these drugs.
Materials and Methods: 24 newly-diagnosed T2D (age 54±2 yrs, BMI 25±1, HbA1c 7.5±0.3%) rendered euglycaemic with i.v. insulin, were studied on two different occasions under the following conditions: (1) Ingestion of a standard mixed meal with no drug administration, and (2) same as (1) but following randomized administration of a single dose of either 120 mg nateglinide (n=12) or 5 mg glibenclamide (n=12) before the meal. Plasma samples for glucose (G), insulin (I), glycerol (GLY), triglycerides (TAG), and non-esterified fatty acids (NEFA), were obtained at standard intervals for 360 min from an arterialized hand vein (A), and from a vein (V) draining the anterior abdominal subcutaneous adipose tissue (AD). Blood flow (BF) in AD was measured with ^{133}Xe. *Calculations*: $NEFA_{Output}=[NEFA_{(V-A)}x(BF)]$, $LPL=[TAG_{(A-V)}x(BF)]$, $HSL=[(GLY_{flux})-(LPL)]$. Postprandial increases are presented as absolute values or areas under the curve (AUC, 0–360 min).
Results: Preprandial levels of G and I in A were comparable in both groups (5.7±0.2 vs 5.9±0.2 mM and 5.0±0.3 vs 6.0±0.4 mU/L respectively). After the meal: (a) the responses of G were similarly reduced by nateglinide (49+3 mM*h) and glibenclamide (41±4 mM*h); (b) secretion of I during the first 30 min was more prominent in the nateglinide (13±1 mU/L*h) than in the glibenclamide group (9±1 mU/L*h, $p<0.05$); in contrast, overall I secretion (0–360 min) was more prominent after glibenclamide (337+30 mU/l*h) than after nateglinide (264±20 mU/l*h, $p<0.05$); (c) TAG levels in A and LPL action were in agreement after nateglinide (4285±436 μmol/l*h and 373+50 nmol glycerol/100 ml_{tissue}*h respectively) and glibenclamide (4983±1004 μmol/l*h and 464±74 nmol glycerol/100 ml_{tissue}*h respectively); (d) NEFA output and HSL action over the first 90 min after the meal were less after nateglinide (167±36 nmol/100 ml_{tissue}*h and 85±10 nmol glycerol/100 ml_{tissue}*h respectively, $p<0.05$) than after glibenclamide (345±33 nmol/100 ml_{tissue}*h and 157+18 nmol glycerol/100 ml_{tissue}*h respectively, $p<0.05$).
Conclusion: The near-physiological insulin secretory profile achieved with nateglinide was more efficient in controlling postprandial hyperglycaemia and in suppressing endogenous lipolysis after the meal, than that achieved with the administration of glibenclamide. These results emphasize the importance of the impairment in insulin secretion pattern in the development of postprandial hyperglycaemia and hyperlipidaemia in type 2 diabetes.

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The second meal effect results from priming of glycogen synthesis in muscle and liver and can be mimicked by administration of arginine

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Background and Aims: The rise in blood glucose after lunch is less if breakfast has been eaten. We have shown that this second meal effect is present in both health and type 2 diabetes. To determine the metabolic basis and potential therapeutic application of the second meal effect we studied 6 healthy subjects on a three separate days.
Material and Methods: Metabolic changes after a lunch, labeled with 3 g of ^{13}C- labeled (99%) glucose, were examined on Day A with breakfast; Day B without breakfast; and day C without breakfast but with intravenous arginine one hour before lunch. Changes in muscle and liver glycogen were measured on Days A and B using magnetic resonance spectroscopy and meal carbohydrate oxidation was measured using breath $^{13}C0_2$ enrichment.
Results: Despite comparable post-lunch insulin and C-peptide increments, the rise in plasma glucose after lunch was significantly less if breakfast had been taken (Day A: 0.8±0.5 mmol/l; Day B: 2.8±0.2 mmol/l, $p<0.02$). Pre-lunch NEFA were suppressed when breakfast had been eaten (0.11±0.02 vs 0.58±0.04 mmol/l). Muscle glycogen storage was greater 5 hours after lunch on a breakfast day (155.3±16.1 vs 40.3±1.9 U $p<0.02$). On the same day the liver glycogen storage was significantly greater 1.5 hours after lunch (171.7±42.8 vs 43.8±18.6 U, $p<0.04$). We observed no difference in carbohydrate oxidation (248.27±107.76 vs 247.98±62.24 U at one hour). Arginine administration brought about a significantly smaller rise in plasma glucose after lunch in these normal subjects (2.1±0.2 vs 2.9±0.2 mmol/l at one hour, $p<0.05$) and this was associated with suppression of pre lunch NEFA (0.21±0.05 vs 0.58±0.04 mmol/l).
Conclusions: The second meal effect is a result of priming of glycogen synthesis in liver and muscle. This phenomenon is associated with sustained suppression of plasma

NEFA. Pre meal administration of arginine mimicks the second meal effect, resulting in lower postprandial glucose rise suggesting a novel therapeutic approach to improving glucose control in type 2 diabetes.
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N-terminal pro brain natriuretic peptide levels increases during weight loss in obese subjects

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Background and Aims: The cardiac hormone brain natriuretic peptide (BNP), including the N-amino terminal (NT-proBNP), are very strong prognostic biomarkers in patients with cardiovascular disease and diabetes. Recent cross-sectional studies have demonstrated that B-type natriuretic peptides are inversely and independently associated with body mass index (BMI), the mechanism behind this relation is unknown. A possible role of both adipose and muscle tissue in clearance and degradation, of these peptides have been suggested. The aims of the present study was to investigate the impact of weight loss on plasma levels of NT-proBNP in obese individuals without heart disease. A second aim was to address the association between NT-proBNP levels, fat mass and lean tissue mass, as measured by DEXA scanning.
Material and Methods: A total of 63 obese individuals, 59 women and 4 men, age 61.7 (±6.6) year, BMI 36.1 (5.5) kg/m^2 (mean±SD) were included. Subjects were without known cardiovascular disease, and NT-proBNP levels were below 125 pg/mL, which is the recommended cut-off value for rule out a diagnosis of heart failure. The participants were randomized 2:1 to either an intervention group, which followed an intensive weight reduction program, including a low energy diet between 3.4 –5 MJ/day for 1 year, or a control group which was offered diet counseling. Body weight, fat mass and lean mass, as well as plasma levels of NT-proBNP were measured at baseline and after 52 weeks.
Results: In the intervention group (n=50) total weight loss was 14.7% (±1.1%) (P<0.001) after 52 weeks, and DEXA scan demonstrated a loss in body fat of 26.1% (±1.9%) (P<0.001), with a decrease in lean mass of 2.4% (±0.7%) (P=0.001) (mean±SE). Plasma NT-proBNP levels increased from 51.6 (39.8–80.1) pg/mL at baseline to 89.1 (49.0–114.9) pg/mL after 52 weeks, (P<0.001) median (interquartile range). In the control group (n=13) a modest weight loss of 4.2% (1.3%) (P=0.003) was observed, the decrease in fat mass was 6.2% (2.3%) (P=0.015), while lean mass was unchanged during follow-up. Decrease in total weight, fat and lean tissue mass were significantly higher in the intervention group as compared with the control group (P<0.001). Furthermore, no change in plasma NT-proBNP levels were observed in the control group 64.9 (25.3–91.1) vs. 55.7 (38.8–118.6) pg/mL (P=0.42). Linear regression analyses demonstrated that higher lean mass was associated with lower NT-proBNP levels r=−0.46 (P<0.001) at baseline, whereas no association between fat mass and NT-proBNP levels were observed (P=0.28).
Conclusions: The present intervention study demonstrate that plasma levels of NT-proBNP increases significantly during weight loss, including both loss in fat and lean mass. Furthermore, the association between higher BMI and lower B-type natriuretic peptide levels may be mediated by lean mass rather than fat tissue mass.

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Oxidative stress and autologous IgG binding to band 3 dimers in erythrocytes of diabetic subjects

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Background and Aims: Oxidative stress is involved in the pathogenesis of long term diabetic complications. Oxidative stress, exacerbated by hyperglycemia, can damage red blood cells (RBCs) given their high content of iron and polyunsaturated fatty acids compared to other cells. Reported abnormalities include altered membrane lipid composition, decreased deformability, increased membrane microviscosity, erythrocyte aggregation, glycated hemoglobin and accumulation of advanced glycation endproducts (AGEs) on the membrane with consequent modifications of rheological properties of RBCs, of their capacity to yield oxygen to tissues and of more rapid RBCs removal from the blood stream by macrophagic system. Our previous studies have shown a link among oxidative stress, intraerythrocyte iron release, alterations of RBC membrane proteins, in particular of band 3, autologous IgG binding and RBC ageing. In the present study we investigated whether diabetic RBCs exposure to oxidative stress results in the binding of autologous IgG to band 3 dimers in a higher percentage of diabetic subjects as compared to health subjects (controls). The iron release and methemoglobin (MetHb) levels in RBCs and the occurrence of total F2-isoprostanes (IsoPs) and of advanced oxidation protein products (AOPPs) in plasma, as markers of oxidative stress, were also studied.

Material and Methods: Our study included 46 diabetic subjects, type 1 and 2 and 20 controls. The IgG binding to band 3 dimers was determined by western blot. The intra-erythrocyte iron release was determined as desferroxamine-chelatable iron (DCI) by HPLC and MetHb by spectrophotometer. F2-isoprostanes were determined by gascromatography-mass spectrometry and AOPPs were measured by absorbance of acetic acid-acidified plasma at 340 nm.
Results: The results showed the presence of IgG binding in 47,8% of diabetics and in 15% of controls (Fisher's Exact Test p=0.0137); no differences between diabetics type 1 and type 2 was observed. MetHb levels were significantly higher in diabetic than in control RBCs ($p<0.001$). DCI was higher in diabetic RBCs showing IgG binding to band 3 dimers, with significant correlation between DCI and IgG binding (optical density obtained by scanning densitometry, $r=0.639$, $p<0.001$). Plasma IsoPs and AOPP levels were significantly higher in diabetic subjects than in controls ($p<0.01$ and $p<0.05$, respectively).
Conclusion: In conclusion our data suggest a link between oxidative stress, iron release and IgG binding to band 3 dimers in RBCs of diabetic subjects. In these subjects a large part of RBCs is ready to be eliminated by macrophagic system, with an accelerated RBCs turnover. The increased erythrophagocytosis and the possible increased exocytosis of iron can have an important role in the LDL oxidation, mechanism underlying atherosclerotic injuries and long term diabetic complications.
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Impact of short term high-fat overfeeding on hepatic glucose production and peripheral insulin action in young healthy men

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Background and Aims: Patients with overt type 2 diabetes (T2D) are characterized by multiple defects of glucose homeostasis with two of the major players being elevated hepatic glucose production (HGP) and peripheral (muscle) insulin resistance. While the relative roles of the liver versus muscle insulin resistance in overt T2D is currently debated, there is a general agreement that muscle insulin resistance is present decades before abnormal HGP and overt T2D in most pre-diabetic states and individuals including first degree relatives, subjects with low birth weight and obese subjects. It has been speculated that insulin resistance in obesity to some extent may be due to the concomitant overeating necessary to maintain an elevated body weight per se. However, little is known concerning the individual role of liver versus muscle in the abnormal glucose metabolism induced by short-term overfeeding in healthy subjects. The aim of this study was to determine the sequential events, and relative roles of liver versus muscle in the putative abnormal glucose homeostasis and insulin action induced by short term overfeeding of a high fat diet in young, lean and healthy subjects.
Materials and Methods: Nineteen healthy 24-year-old, non-obese male volunteers without a family history of diabetes were included in the study. The subjects were randomized to two study periods separated by 4–6 weeks; a 5-day period on a high-fat (60E%), high-energy (+50%) overfeeding-diet, and a control period on an iso-energetic control diet. At the end of each period, the participants were studied using the hyperinsulinemic euglycemic clamp technique (80 $mU/m^2/min$) combined with indirect calorimetry and a primed, continuous infusion of [3-^{3}H] glucose to study the partitioning of glucose metabolism between liver and periphery. Intravenous glucose tolerance tests were performed prior to the clamps to measure first phase insulin secretion.
Results: Five days of high-fat overfeeding resulted in an increase in the mean fasting blood glucose levels (4.59±0.44 vs. 5.00±0.48 pmol/L, P=0.01). Furthermore, fasting (basal) HGP was increased by overfeeding (2.05±0.33 vs. 2.58±0.32 mg/kgFFM/min, P=0.01). HGP was fully suppressed during insulin infusion both before and after overfeeding, and overfeeding did surprisingly not influence peripheral (muscle) glucose disposal (14.05±2.37 vs. 13.96±3.42 mg/kgFFM/min, P=0.86). First phase insulin secretion capacity as well as basal and insulin stimulated oxidative- and non-oxidative glucose metabolism was not significantly influenced by overfeeding.
Conclusion: Short term overfeeding increases fasting plasma glucose levels in healthy, young men. This effect is partly - or fully - mediated by a rise in basal hepatic glucose production. In contrast, insulin secretion capacity as well as peripheral insulin action was not significantly affected by overfeeding. The data suggest that elevated HGP in overt T2D patients to some extent may be due to overeating. In contrast, peripheral insulin resistance in obese subjects or in patients with overt T2D, is caused by factors other than short term overfeeding per se.
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High fat and lipid induced insulin resistance in rats: the comparison of glucose metabolism, plasma resistin and adiponectin levels

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Background and Aims: In animal models, both acute elevation of plasma FFA via intravenous infusion of lipid emulsion and chronic elevation of plasma FFA via high-fat feeding have been shown to induce skeletal muscle and liver insulin resistance. However, very few studies have compared the effects of high fat and lipid induced insulin resistance on glucose metabolism and adipocytokine in the same experiments *in vivo*. In the current study, we used a lipid infusion and high fat feed in combination with hyperinsulinemic–euglycemic clamps to assess the impact of acute and chronic elevated FFA levels on overall glucose metabolism and insulin action, two adipocytokines resistin and adiponectin.
Materials and Methods: Male S–D rats were divided into 3 groups: control(n=10), HF(high fat diet,n=10), lipid groups(lipid emulsion was infused, n=10). Five to seven days before the study, rats receivered a surgery. A silastic catheter was inserted into the right internal jugular vein and extended to the level of the right atrium. Another catheter was advanced through the left carotid artery until its tip reached the aortic arch. After an overnight fast, euglycemic–hyperinsulinemic clamps were performed with awake and unrestrained rats. glucose concentrations were clamped at euglycemic levels. 3-^{3}H glucose was infused through the jugular vein catheter starting at 60 min. A lipid emulsion was infused at a rate of 5 ml/kg/h after 60 min(lipid group). Controls and high-fat group (HF group) received saline infusions instead of lipid. Insulin was infused from 180 to 300 min. Blood samples were obtained from the carotid artery at 0, 60, 260, 280, 290 and 300 min for determination of insulin, resistin, adiponectin, free fatty acids and specific activity of tritiated glucose.
Results: In baseline, plasma free fatty acids (FFA) levels were significantly increased in high fat rats (HF group) compared with controls(P<0.05). During clamp steady-state, the FFA levels were reduced by ~77 and ~56% in control and HF groups, respectively, but there was a significant increase in plasma FFA level in lipid-infused rats (lipid group).The glucose infusion rates (GIR) in HF and lipid groups were obviously lower than in the controls (all P<0.01). Moreover,GIR was lower in lipid group compared with HF group (P<0.05). The rate of glucose disappearance (G_{Rd}) was significantly lower in lipid group compared with controls. The endogenous glucose production (EGP) in controls was suppressed by ~85%, but only suppressed by ~29 and ~19% in HF and lipid groups, respectively. The resistin levels of muscle tissues in Lipid group was significantly higher compared with control and HF groups (Both P<0.05). After the insulin clamp, circulating adiponectin levels were significantly decreased in the lipid group compared with the control and HF rats. (P<0.05).
Conclusion: Both lipid infusion and high fat feed can induce peripheral and hepatic insulin resistance in rats, and the former was more significantly, it might be associated with elevated resistin.

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Low efficiency of n-3 polyunsaturated fatty acids in the reversal of insulin resistance induced by a high-fat diet in mice

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Background and Aims: Previous studies including ours established that n-3 polyunsaturated fatty acids (n-3 PUFAs) from marine fish oils, potent natural hypolipidemics, could prevent obesity and insulin reistance (IR) induced by high-fat (HF) diets in laboratory rodents while counteracting proliferation of adipose tissue cells. Considerably less is known about the efficiency of n-3 PUFAs in the reversal of IR associated with an already established obesity. Therefore, we analyzed the effects of n-3 PUFAs, namely eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, in the obese C57BL/6N (B6) mice.
Materials and Methods: Male B6 mice, fed a standard chow from weaning, were assigned a HF diet (60% calories from fat, mainly corn oil) at the age of 3 mo. Obesity was induced during 3 mo of HF feeding (mean body weigth ~46.3±0.8). At this stage, some mice were randomly assigned to a HF diet, in which 9% of dietary lipids was replaced by EPA and DHA, added as a 60% concentrate (EPAX1050TG; Pronova Biocare, Norway), while the rest of mice was maintained on a HF diet (n=10). Body weight gain and food intake were monitored during 2 mo of feeding. No differences in food intake were noted. Intraperitoneal glucose tolerance test (IPGTT; D-glucose as a bolus of 1 mg/g body weight) was used to assess glucose tolerance before and after EPA/DHA feeding. Plasma lipids were measured by enzymatic methods, insulin by RIA (Linco), and blood glucose by glucometers (OneTouch Ultra; LifeScan). DNA content in epididymal fat was measured by fluorometry. Statistical analysis was performed using One way ANOVA or t-Test.

Results: Compared to mice maintained on a HF diet, 2 mo of EPA/DHA feeding resulted in a significantly lower body weight gain (5.2±0.3 vs. 2.4±0.9 g) and plasma levels of triacylglycerols (2.00±0.29 vs. 1.21±0.17 mmol/L), non-esterified fatty acids (0.70±0.05 vs. 0.44±0.03 mmol/L), and cholesterol (5.58±0.26 vs. 4.24±0.23 mmol/L) in the fed state. Differences in fasted blood glucose (103±6 vs. 89±5 mg/dL) were of borderline significance (P=0.07). Fasted plasma insulin, blood glucose and plasma insulin in the fed state, as well as weights of the liver, white subcutaneous and epididymal fat, and interscapular brown adipose tissue did not differ between the groups. Cellularity of epididymal fat, assessed as a DNA content, did not change. Comparison of IPGTT data obtained before and after the treatment with EPA/DHA showed significant improvements in blood glucose levels during the first 60 min of the test, while no significant changes were observed in the control group maintained on a HF diet.
Conclusion: In contrast to a potent effect of EPA and DHA in the prevention of HF diet-induced obesity and IR, these n-3 PUFAs exerted only modest effects on body weight and glucose tolerance once obesity and IR had been already established. However, the strong lipid-lowering effect was retained even in the obese animals. The potency of EPA and DHA to affect adiposity and insulin sensitivity during the development or reversal of obesity correlates with their differential effect on cellularity of white adipose tissue. Prevention of adipose cell proliferation by EPA and DHA may be essential for the potent and beneficial effects of these n-3 PUFAs on adiposity and insulin sensitivity.

PS 36

Other hormones I

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Angiotensin II modulates Na^+/K^+ ATPase subunits in diabetic rat kidney

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Background and Aims: Angiotensin II (AII) is synthesized in the kidney and is a progressive mediator of renal injury in diabetic nephrosclerosis. Previous studies demonstrated that AII directly stimulates Na^+/K^+ATPase (NKA) activity via angiotensin 1 receptor in the proximal tubules, thereby affecting blood pressure and contributing to hypertension. The clinical efficacy of angiotensin II receptor blockers has been widely implicated in the therapy of diabetic nephropathy, but the exact mechanism is still unknown. Thus we investigated the direct effects of AII treatment on renal Na^+/K^+ ATPase, a key enzyme of kidney function in diabetic rats. The present study evaluates renal mRNA, protein expression, phosphorylation and enzyme activity of NKA α-1 and β-1 subunits in AII treated streptozotocin (STZ) diabetic rats.
Materials and Methods: Six-week-old, male Wistar rats control (C) and STZ (65 mg/kg) induced diabetic after 3 weeks (D3) and 7 weeks (D7) from the onset of diabetes were divided into diabetic and insulin treated (2 times daily 2–4 U insulin (Humulin U Lilly) according to the blood glucose levels), reverted diabetic (D3R and D7R) groups. All five groups were further subdivided into untreated and AII treated (24 h, 33 μg/kg/h via osmotic pump) subgroups (CA, D3A, D7A, D3AR, D7AR). The minipump was implanted under anesthesia (40 mg pentobarbital sodium/ kg ip.) Kidney cortex was homogenized and specific mRNA levels were quantified by RT-PCR. The protein expression of the α1 and the β1 subunits of NKA as well as the phosphorylation status were analyzed using Western blotting technique and specific antibodies. The enzyme activity was measured in a coupled reaction. The data were analyzed on STATISTICA.6 software (StatSoft® Inc., USA). Data are presented as means±S. D, and were tested for normal distribution with Kolmogorov–Smirnov test. Criterion for significance was $P<0.05$ in all experiments.
Results: In D7 rats the mRNA expression and protein level of α1 subunit of NKA was significantly elevated. This change was not observable in D3 animals. Administration of insulin reverted the changes observed in the D7 animals by lowering the level of the α1 subunit mRNA and protein. AII administration alone elevated the mRNA and protein expression of the α1 subunit in C, D3 and in D7 animals. This effect was less pronounced when the diabetes was reverted with insulin. A rise in the mRNA and protein levels of the β subunit of NKA was also observable in reponse to AII treatment, but to a lesser extent. Both diabetic condition and AII treatment increased NKA enzyme activity measured in D7 rats, while D3 rats did not exhibit the same change in enzyme activity. Both diabetic condition and AII administration changed the serin phosphorylation level of the α1 subunit.
Conclusion: Diabetes and AII administration both elevate the level of mRNA and protein of the α1 subunit and increase the activity of NKA. AII effect is more pronounced, when the animal suffers from long term (D7) untreated diabetes. Diabetic condition seems to potentiate the effect of AII on the activity of NKA. This might

contribute to the development of hypertension in diabetes mellitus and explain the beneficial effect of AII blockers.

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Prolactin modulates the association and phosphorylation of SNARE and kinesin/MAP-2 proteins in neonatal rat islets

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Background and Aims: During pregnancy and the perinatal period of life, prolactin induces maturation of the stimulus-secretion coupling mechanism in pancreatic β-cells. However, the mechanisms of mobilization of vesicles as well as the extrusion of the insulin containing granules are not yet completely understood. We investigated here whether the improved secretory response to glucose by prolactin, involves alteration in the association and phosphorylation of several proteins that participate in these processes.
Materials and Methods: The association and phosphorylation of proteins was analyzed by immunoprecipitation followed by Western blotting, after acute exposure to prolactin.
Results: Association between SNARE proteins and kinesin/MAP-2 was increased by prolactin while the association of munc-18/syntaxin 1A was decreased. Serine phosphorylation of SNAP-25, syntaxin 1A, munc-18, MAP-2 was significantly higher while kinesin phosphorylation was decreased in prolactin-treated islets. We observed an increase in SNARE complex formation in islets stimulated by prolactin, 22 mM glucose, 40 mM of K^+, Carbachol and PMA. The prolactin-induced increase in the formation of SNARE complex and syntaxin 1A phosphorylation was inhibited by PD098059, a blocker of MAPK pathway.
Conclusion: These findings indicate that prolactin prime the pancreatic β-cells to release insulin by increasing the phosphorylation/association of proteins implicated in the secretory machinery, probably involving the MAPK/PKC pathway.
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Splanchnic cortisol production in dogs occurs primarily in the liver with only a small contribution from viscera and visceral fat: **Evidence for tissue specific 11β hydroxysteroid dehydrogenase type 1 activity**

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Background and Aims: Glucocorticoids are potent regulators of protein, fat and carbohydrate metabolism. We have shown in humans that the splanchnic bed produces cortisol at rates approximating extra-adrenal tissues by converting cortisone to cortisol via the 11 β-hydroxysteroid dehydrogenase type1 (11 β-HSD-1) pathway. Since portal vein catheterization is invasive and impractical in humans and *in vitro* studies indicated that 11 β-HSD-1 activity is present in both visceral fat and liver, the present studies were undertaken to determine the relative contribution of the viscera and the liver to splanchnic cortisol production in the dog.
Materials and Methods: Seven dogs underwent the combined hepatic/portal vein catheterization method and an infusion of cortisol (F) labeled with 4 deuteriums (4DF). Total body, portal and hepatic D-3 cortisol production (an index of 11 β-HSD-1 activity) were measured.
Results: Hepatic artery (HA), portal vein (PV) and hepatic vein (HV) cortisol concentrations did not differ (0.33±0.12 vs. 0.31±0.11 vs. 0.29±0.10 μg/dl) indicating zero net cortisol balance. However, 4DF concentrations progressively decreased ($p<0.0001$ ANOVA) from the HA to PV to HV (2.6±0.09 vs. 2.4±0.08 vs. 1.7±0.06 μg/dl) resulting in 8.0±2.0% and 30.3±2.0% extraction across the viscera and liver indicating 0.07±0.03 and 0.33±0.03 μg/min cortisol uptake ($p<0.04$ and $p<0.0001$ vs. zero ; $p<0.0002$ vs. one another). Since net cortisol balance was zero, this meant that the viscera was producing cortisol at a rate of 0.02±0.01 μg/min ($p<0.04$ vs zero) and the liver 1.0±0.0 μg/min ($p<0.001$ vs. zero; $p<0.001$ vs. viscera). 4DF loses a deuterium during conversion via 11-β HSD type 2 to 3D cortisone (3DE) which in turn yields 3DF when 3DE is converted to cortisol via 11-β HSD type 1. Therefore 3DF production provides an index of cortisone to cortisol conversion via the 11-β HSD type 1 pathway. 3 DE concentrations did not differ in PV and HA (0.46±0.04 vs. 0.40±0.06 μg/dl) but were markedly lower ($p<0.002$) in the HV (0.02±0.002) indicating extensive uptake of the precursor cortisone by the liver but not by the viscera. 3DF concentrations were lower ($p<0.01$) PV vs. HA (0.45±0.03 vs 0.49±0.02) but did not differ in the HV indicating net uptake across the viscera but zero balance across the liver. 3DF uptake averaged 0.01±0.00 indicating a visceral 3DF production rate of 0.02±0.01 ug/min ($p<0.05$ vs. zero). In contrast, 3DF uptake across the liver averaged 0.08±0.01 μg/min indicating liver 3DF production rates of 0.09±0.01 μg/min ($p<0.0001$ vs. zero). 3DF production was greater ($p<0.0002$) in the liver than the viscera indicating

greater flux via the 11-β HSD type 1 pathway. Total body cortisol production rates in extra splanchnic tissues (e.g. the adrenals) averaged 1.7±0.2 μg/min.
Conclusions: We conclude that in dogs a) the viscera and liver both take up cortisol with extraction being greater in the liver, b) small amounts of cortisol may be made by the viscera (D3F data), however c) in excess of 80% of splanchnic cortisol production occurs within the liver suggesting a potentially important role in the regulation of hepatic glucose, fat and protein metabolism.
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Progesterone-induced inhibition of glucose utilisation in rat skeletal muscle: involvement of a receptor located on the cell surface?

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Background and Aims: Late pregnancy is characterised by loss of insulin sensitivity, which can lead to deterioration of glucose homeostasis and gestational diabetes. This has been attributed, at least in part, to increased circulating concentrations of progesterone. The present study was to examine, if and how progesterone directly affects glucose metabolism of skeletal muscle, which is the predominant tissue for insulin-induced glucose uptake.
Materials and Methods: Freshly isolated rat soleus muscle strips were incubated with progesterone or other steroids for 4 h and insulin-stimulated (25 nmol/l) rates of glucose metabolism were measured during the subsequent hour. Furthermore, the expression of a progesterone binding protein in skeletal muscle was examined by semiquantitative PCR using specific primers.
Results: Exposure to 1 μM progesterone, a concentration reached in plasma of pregnant rats and humans, impaired insulin-stimulated glycogen synthesis in isolated rat muscle (−14±5%, $p<0.01$; all results given as % change vs. intraindividual control), which resulted in decreased muscle glycogen content (−13±5%, $p<0.02$). This became more distinct at higher concentrations of progesterone with an almost complete suppression of glycogen storage by 50 μM progesterone (glycogen synthesis: 3 μM, −33±6%; 10 μM, −73±3%; 50 μM, −87±6%; glycogen content: 3 μM, −28±5%; 10 μM, −52±4%; 50 μM, −91±7%; $p<0.001$ each). Impaired glycogen storage was accompanied by reductions in the rates of glucose transport (50 μM progesterone; −15±4%; $p<0.005$) and glucose oxidation (−80±3%; $p<0.001$). Pharmacological inhibitors of transcription, translation, or the cytosolic progesterone receptor failed to block progesterone-induced impairments of glycogen synthesis (effect of 50 μM progesterone in the presence of 1 mg/l actinomycin D, −61±10%; of 1 mg/l cycloheximide, −68±7%; and of 10 μM RU486, −83±2%; $p<0.001$ each) and glucose oxidation (−52±10%, −53±4%, and −79±3%, respectively; $p<0.001$ each), suggesting that progesterone acted via a non-genomic mechanism that could involve a receptor located on the cell surface. Two progesterone binding proteins on the cell surface have been described and are regarded as mediators of non-genomic progesterone actions (i.e. putative progesterone receptors). Whereas one of them is not expressed in skeletal muscle, we found that the other, which is believed to mediate the progesterone-induced acrosome reaction of sperm, is abundant in rat skeletal muscle. The relative potentials of various steroids to inhibit glycogen synthesis showed similarities with their reported binding activities to this progesterone binding protein. This included that testosterone was a relatively potent inhibitor of glycogen synthesis (−61±6%, $p<0.001$) while dexamethasone was rather weak (−13±8%, ns), and that corticosterone (−36±4%, $p<0.001$) was clearly superior to cortisol (−12±6%, $p<0.01$; steroids compared at 50 μM).
Conclusion: Progesterone distinctly reduces glucose utilisation by isolated rat skeletal muscle via a non-genomic mechanism of action. This mechanism could contribute to impaired glucose homeostasis in late pregnancy and could serve to shift more fuel substrate towards the fetus. Furthermore, our findings make it tempting to speculate that this effect could be mediated by a progesterone binding protein located on the cell surface, which also mediates the acrosome reaction of sperm.

0571

Physiological concentrations of C-peptide increase Na, K-ATPase expression via PKC- and MAP kinase-dependent activation of transcription factor ZEB in human renal tubular cells

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Background and Aims: Accumulating evidence indicates that replacement of proinsulin C-peptide in type 1 diabetes ameliorates nerve and kidney dysfunction. These disorders are associated with decreases in Na,K-ATPase activity. C-peptide shows specific binding to a G-protein coupled membrane receptor resulting in Ca^{2+} influx, activation of a PKC and

MAP kinase signaling pathways and stimulation of Na,K-ATPase activity. We determined the molecular mechanism by which long-term exposure to C-peptide stimulates Na,K-ATPase expression and activity in primary human renal tubular cells (HRTC) in normo- and hyperglycemic conditions.

Materials and Methods: Human renal tubular cells were cultured from the outer cortex of renal tissue obtained from patients undergoing elective nephrectomy. Cells were cultured in 5 mM or 25 mM glucose in absence or in presence of 1 nM or 10 nM of C-peptide. Ouabain-sensitive rubidium ($^{86}Rb^{+}$) uptake and Na,K-ATPase activity were determined. Abundance of Na,K-ATPase was determined by Western blotting in intact cells or isolated basolateral membranes (BLM). DNA binding activity of transcription factors was determined by electrical mobility shift assay (EMSA).

Results: Culturing of HRTCs with 1 nM, but not 10 nM, of human C-peptide for 5 days resulted in 62% ($p<0.05$) increase in Na,K-ATPase α_1-subunit protein expression, accompanied by 37% ($p<0.05$) increase in $^{86}Rb^{+}$ uptake, both in normo- and hyperglycemic conditions. High glucose concentrations did not affect Na,K-ATPase α_1-subunit expression but Na,K-ATPase activity was reduced in BLM isolated from cells cultured in presence of high glucose in a PKC-dependent manner, suggesting that during hyperglycemia Na,K-ATPase may have internalized and sequestered in intracellular stores. Exposure to high glucose resulted in significant ($p<0.01$) increase in phosphorylation of ERK1/2, PKCα/β and decrease in IκBα abundance in the absence or presence of C-peptide. In parallel with augmented α_1-subunit expression and stimulation of ouabain-sensitive $^{86}Rb^{+}$ uptake, exposure to 1 nM of C-peptide increased DNA binding activity of ZEB (AREB6), a transcription factor involved in regulation of Na,K-ATPase α_1-subunit expression. ZEB DNA binding activity was not affected by exposure to 10 nM C-peptide or high glucose concentrations. Effects of 1 nM C-peptide on Na,K-ATPase α_1-subunit expression and ZEB DNA binding activity in HRTC were abolished by overnight incubation with PKC inhibitor (1 μM GF109203X) or MEK1/2 inhibitor (10 μM PD98059), suggesting involvement of conventional and novel PKCs and ERK1/2 in the regulation C-peptide induced sodium pump expression.

Conclusion: Our results indicate that C-peptide in physiological concentration stimulates sodium pump expression via activation of ZEB, a transcription factor not previously implicated in C-peptide-mediated signaling. The results suggest that despite activation of ERK1/2 and PKC by hyperglycemia, C-peptide influences a distinct pool of PKCs and ERK1/2 in the regulation of Na,K-ATPase expression and activity.

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0572

Mechanisms by which the Amylin analogue pramlintide reduces postprandial glucose (PPG) excursions in humans

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Background and Aims: Pramlintide helps to reduce PPG excursions by slowing gastric emptying (GE). PPG increases occur due to increases in the rate of glucose appearance. The latter is the sum of endogenous glucose production and the appearance of ingested glucose not been sequestered by the liver. Thus PPG reduction could result from greater suppression of endogenous glucose production or greater meal splanchnic sequestration

Materials and Methods: Nine healthy subjects were studied (4M, 5F, 41 3 y, BW 84 3 kg, BMI 27.8±1.1 kg/m^2). After 3-h primed continuous 13-C glucose infusion, 30 g pramlintide or placebo (PBO) were injected sc and subjects ate a mixed meal, 50 g glucose, 25 g fat and 20 g protein, enriched with 3 g 6,6-dideuteroglucose. Also, the meal was labelled to measure GE scintigraphically.

Results: Starting with comparable glucose conc. (4.5±0.1 vs 4.4±0.1 mM, ns, PBO vs verum), PPG excursions were greater over the initial 120 min with PBO (5.2±0.1 vs 4.8±0.1 mM, $p<0.001$ PBO vs verum). Insulin concentrations followed a similar pattern and were greater in PBO (48.0±4.3 vs 23.9±4.2 U/ml, $p<0.001$).

No differences in glucagon were found (53.7±4.7 vs 52.8±5.2 pg/ml, ns). Despite lower PP insulin, PPG excursions were lower in the verum, corresponding to significant reduction in total plasma glucose appearance (24.6±1.3 vs 18.5±1.4 mol/kg/min, $p=0.007$). Endogenous glucose trended lower with PBO (6.2±0.6 vs 8.7±0.9 mol/kg/min, $p=0.05$). Slowed GE in the verum arm (67.6±3.1 vs 78.2 2.7 retention at 120 min $p<0.05$) led to a reduction in the rate of appearance of the oral glucose (18.4±1.2 vs 9.9±1.5 mol/kg/min, $p<0.05$) consistent with greater splanchnic sequestration of the meal despite lower insulin concentrations. Pramlintide was well tolerated.

Conclusion: Pramlintide reduced PPG excursions by slowing GE which led to greater splanchnic sequestration of the meal reduced pp plasma glucose concentrations.

Supported by Amylin Pharmaceuticals, Inc

0573

Nicotinic receptors on pancreatic beta cells are regulated by nicotine and glucose

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Background and Aims: We previously documented for the first time in beta cells specific binding and mRNA expression for different subunits of acetylcholine nicotinic receptors of the neuronal type (nAchr). We have presently tested for protein expression, regulation and functional importance.
Materials and Methods: Rat pancreatic islets and clonal INS-1 cells were used.
Results: Western immunoblotting detected strong signals for the alpha 7 subunit of the nAchr. both in pancreatic islets and INS-1 cells. Culture at 27 vs. 5.5 mM glucose down-regulated the protein expression by 81+/− 3 percent, $p<0.001$. Exposure to nicotine (10–6M) for 48 h up-regulated the alpha7 protein by 19+/−5 percent, $p<0.01$. Mecamylamine which inhibits nAchr. reduced the viability of INS-1 cells 92.2 percent by MTT assay. This negative effect was abolished by pre-exposure to nicotine. On the other hand pre-exposure to nicotine did not affect the impact of toxicity tested by exposure to H_20_2, 400 mikroM, or Interleukin-1beta 0.5 ng/ml for 24 h in INS-1 cells.
Conclusion: Subunits of nAchr are strongly expressed and regulated in beta cells. Activation of nAchr may play a positive role for viability of beta cells.

0574

The peroxisome proliferator-activated receptor -α and γ agonists modulate regulation and metabolism of glucocorticoids through different mechanisms

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Background and Aims: Glucocorticoid (GC) is one of main hormonal antagonistics to the physiological function of insulin *in vivo* and executes its function by glucocorticoid receptor (GR). The increased GC concentrations in circulation or at local tissues have been found closely related with insulin resistance. 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) converts inactive GCs to active GCs at local tissues and the increased gene expression and enzyme activity of 11β-HSD1 especially in adipose tissue have been found associated with obesity and insulin resistance. The peroxisome proliferator-activated receptor (PPAR)-α and -γ agonists are widely used for their roles on regulating lipid and glucose homeostasis. Some researches suggested both PPAR-α and -γ agonists could downregulate 11β-HSD1 gene expression in liver and adipose tissue respectively. Therefore, this study was designed to investigate whether the metabolism of GCs at pre-receptor and receptor levels took part in the development of insulin resistance induced by high fat and high starch diets and whether PPAR-α and -γ agonists were involved in the metabolism and regulation of GCs.
Materials and Methods: We observed that gene expression of 11β-HSD1 was increased in liver and adipose tissue in rats fed on high fat and high starch diets and was attenuated by PPAR-γ agonists Pioglitazone and PPAR-α agonist Fenofibrate.
Results: To our surprising, we first found that Fenofibrate could increase GR isoforms GRβ/GRα protein ratio especially in liver tissue resulting in glucocorticoid resistance at receptor level and leading to increased circular corticosterone level. We concluded that the increased expression level of 11β-HSD1 was associated with insulin resistance and PPAR-α and PPAR-γ agonists modulated regulation and metabolism of glucocorticoids through different mechanisms.
Conclusion: We first discovered that Fenofibrate could induce glucocorticoid resistance and a complex feedback loop may exist between PPAR-α and glucocorticoid receptor.

PS 37

Other hormones II

0575

Postprandial hyperglucagonaemia contributes to hyperglycaemia in somatostatin receptor subtype 2 deficient mice with diet-induced obesity and insulin resistance

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Background and Aims: Somatostatin (SST) inhibits glucagon and insulin secretion. Five receptor subtypes for SST are known (SSTR1-SSTR5), all of which are expressed in the endocrine pancreas. SSTR2 inhibits glucagon secretion *in vitro*. Glucagon increases blood glucose levels through increased hepatic glucose production. Excessive circulating glucagon increases the rate of

hepatic gluconeogenesis in type 2 diabetes, thereby contributing to hyperglycemia. Although the role of SSTR2 at inhibiting glucagon secretion has been demonstrated *in vitro*, there are no studies characterizing the role of SSTR2 in type 2 diabetes.

Here, we characterize the role of SSTR2 in regulating glucose homeostasis in a mouse model of type 2 diabetes.

Materials and Methods: Male SSTR2-deficient ($SSTR2^{-/-}$) and age matched control mice ($SSTR2^{+/+}$) were fed high fat diet (HFD) (60% kcal w/w) for 14 weeks and the parameters of glucose homeostasis were monitored. Hepatic glycogen and lipid content was quantified enzymatically and visualized by histomorphology. RNA levels of enzymes regulating glycogen synthesis and breakdown were measured by a real time PCR and their activity by Western blot. Insulin, somatostatin and glucose tolerance tests were performed. Glucagon secretion from isolated islets was measured by RIA.

Results: Despite a similar body weight gain in response to HFD, the weight of the visceral fat of SSTR2-deficient mice was decreased. Postprandial glucagon, glucose as well as fasting glucose, and free fatty acid levels were increased in SSTR2-deficient mice. Glucose disappearance rate following ip administration of glucose, insulin or SST was delayed in $SSTR2^{-/-}$ mice. In the postprandial state, SSTR2-deficient mice had decreased hepatic glycogen content. Furthermore, SSTR2-deficient mice had decreased mRNA levels of glucokinase. The activity of glycogen synthase of $SSTR2^{-/-}$ mice was decreased while glycogen synthase kinase-3 was increased. The phosphorylation state and mRNA levels of glycogen phosphorylase, phosphorylase-kinase, and CREB were increased. The hepatic lipid content of SSTR2-deficient mice was decreased. Glucose less potently suppressed glucagon secretion from islets isolated from SSTR2-deficient mice.

Conclusion: This study for the first time demonstrates the SSTR2 dependent regulation of glucagon secretion in mouse model of type 2 diabetes. Deletion of SSTR2 accounts for the postprandial hyperglucagonemia. Increased glucose concentration may be due to decreased hepatic glucose utilization, lipid accumulation, and increased glycogen breakdown. SSTR2 may provide a valuable therapeutic target at improving hyperglycemia in type 2 diabetes.

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0576

EGR-1 and CREB are target molecules for orexin–A dependant inhibition of glucagon gene expression

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Background and Aims: Glucagon plays a major role in regulating glucose homeostasis. Recently it has been demonstrated that the early growth response-1 transcription factor (egr-1) upregulates glucagon gene expression in response to treatment with gastrin. Orexin-A (OXA) is a hypothalamic neuropeptide that reduces glucagon secretion *in vivo*, however the effects of OXA on pancreatic A-cells on the cellular level have not yet been demonstrated. Aim of our study was therefore to characterise the effects of OXA on glucagon producing cells (InR1-G9) and the underlying signal transduction pathway.

Materials and Methods: OXR-1 expression in InR1-G9 cells was detected by western blot and the intra-cellular distribution pattern by immunofluorescence. Intracellular concentration of cAMP, the activity of cAMP response element binding protein B (CREB) and egr-1 were measured by ELISA, western blots and real-time PCR. Changes of the intracellular calcium (Ca^{2+}) were monitored by Fura-2 measurements.

Results: InR1-G9 cells express OXR-1 on mRNA and protein level. OXR-1 was co-localized with glucagon at the cytoplasmatic compartment. OXA reduced glucagon secretion and proglucagon gene expression. Short time OXA treatment (up to 20 min.) decreased intracellular cyclic AMP as well as Ca^{2+} levels. OXA reduced CREB mRNA and dephosphorylated CREB protein. Furthermore, OXA reduced the expression of egr-1.

Conclusion: Our study provides the first *in vitro* evidence for OXA as an inhibitor of glucagon secretion and gene expression. In addition we identify cAMP and Ca^{2+} as intracellular target molecules for OXA action. Furthermore our results demonstrate two different transcription factors CREB and egr-1 that act as downstream targets for OXA signaling, suggesting a role in mediating the inhibitory effects of OXA on glucagon gene expression. We have now increasing evidence that OXA affects glucagon homeostasis. The inhibition of glucagon biosynthesis and secretion by OXA may provide a potential use in therapy of glucagon hypersecretion in type 2 diabetes.

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Orexin-A inhibits glucagon secretion and gene expression by foxo1 dependant pathway

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Background and Aims: Orexin-A (OXA) regulates food intake and energy homeostasis. In vivo, OXA increases insulin secretion and inhibits glucagon secretion, suggesting a role of OXA in regulating glucose homeostasis. However, it is not known whether the effects of OXA are mediated through a direct interaction with the pancreatic A-cells. Aim of the study was therefore to provide the evidence for the direct interaction of OXA with glucagon producing cells, describe the biological effects, and characterize the underlying intracellular mechanisms.
Materials and Methods: The effects of OXA on glucagon secretion were evaluated using an in situ perfused rat pancreas model and clonal pancreatic A-cells (InR1-G9). Glucagon was measured by RIA. OXR-1 expression in InR1-G9 cells was detected by western blot and immunofluorescence. The effects of OXA on intracellular cyclic AMP, AKT, PDK-1 were measured by ELISA and western blots, changes of the intracellular calcium (Ca^{2+}) by Fura-2 measurements. Proglucagon and foxo1 mRNA levels were quantified by real-time PCR. Silencing of foxo1 in InR1-G9 cells was performed by transfection with short interfering RNA (siRNA).
Results: OXR-1 expression in InR1-G9 cells was detected by western blots and confirmed by immunofluorescence. OXA inhibited glucagon secretion from perfused rat pancreas and InR1-G9 cells, and proglucagon gene expression in InR1-G9 cells. OXA decreased intracellular cyclic AMP and Ca^{2+} concentrations, and increased the phosphorylation of AKT und PDK-1. PI-3 kinase inhibitor blocked the effects of OXA on proglucagon gene transcription. Silencing of foxo1 had no effects on basal proglucagon gene expression; however the inhibitory effect of OXA on glucagon gene expression was reversed.
Conclusion: Our study provides the first *in vitro* evidence for the interaction of OXA with pancreatic A-cells. OXA inhibits glucagon secretion and proglucagon gene expression. In addition we identify cAMP/AKT/PDK-1 and Ca^{2+} as intracellular target molecules for OXA action. Furthermore, we demonstrate that foxo1 mediates the inhibition of proglucagon gene expression in response to OXA treatment. Inhibition of glucagon secretion by OXA may have potential implication at lowering hyperglucagonemia frequently encountered in type 2 diabetes.

0578

Metabolic evaluation of FGF-21 transgenic mice

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Background and Aims: Fibroblast Groth Factor 21 (FGF-21) is a secreted 20 KDa protein and a novel member of the FGF family that is predominantly expressed in the liver and to a lower extent in the asinar cells of the pancreas. While the majority of the 22 known members of FGF family have been primarily associated with mitosis, transformation, angiogenesis, and survival, recent data shows that they may play important roles in defining and regulating functions of some endocrine-relevant tissues and organs, as well as modulating various metabolic processes. We have recently discovered FGF-21 as a potent regulator of glucose homeostasis.
Materials and Methods: To further investigate the FGF-21 biology we generated and explored FGF-21 transgenic mice.
Results: FGF-21 transgenic mice that express the protein from the liver are viable, lean, resistant to diet induced obesity, and overall have a favorable metabolic phenotype.
Conclusion: While these mice have a well characterized phenotype, little is known about the underlying mechanisms by which FGF-21 over-expression modulates metabolism. Indirect calorimetry and extensive gene expression data of several important metabolic tissues will be presented that provide further insight in the biology of FGF-21.

0579

Effect of acute hyperinsulinaemia and AT-1 receptor blocade on activity of 5α-reductase and aromatase in healthy subjects and subjects with type 2 diabetes

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Background and Aims: Several components of renin-angiotensin system, including AT-2 and AT-1 receptor were detected within the testis. Hyperinsulinaemia/insulin resistance is another factor interacting with steroidogenesis. Connection between lower testosterone levels and type 2 diabetes in men was delineated, however the effect of hyperinsulinaemia and acute AT-1 receptor blockade on testosterone metabolism *in vivo* was not investigated till now. The aim of the study was to study the effect of acute hyperinsulinaemia with and without acute AT-1 receptor

blockade (AT) on the activity of 5α - reductase and aromatase in men with type 2 diabetes (D) and healthy subjects (H).
Materials and Methods: Serum concentrations of testosterone (T), dihydrotestosterone (DHT) and estradiol (E2) were measured before and at 240 minutes of hyperinsulinaemic (1 $mU.kg^{-1}.min^{-1}$) euglycaemic (5 $mmol.l^{-1}$) clamp (HIC), and during HIC after acute AT-1 receptor blockade (cumulative dose of losartan was 200 mg) using the same protocol (AT-HIC) in 8 H and in 11 D. Enzymatic activities were computed as product/precursor ratios (DHT/T for 5 alpha reductase (5αR) and E2/T for aromatase (A)). ANOVA with repeated measures, followed by Bonferroni multiple comparisons were used.
Results: D had significantly lower insulin sensitivity than H, as measured by metabolic clearance rate of glucose (MCR) ($p<0.001$), without any significant changes during AT-HIC in either group. DHT was lower in D than in H ($p<0.02$) and declined during HIC ($p<0.001$) in all subjects; the decrease was more pronounced in H ($p<0.006$; ANOVA). The AT increased the DHT levels ($p<0.01$) in all subjects. 5αR activity was lower in D than in H ($p<0.006$) and declined after HIC ($p<0.001$). The decrease was more pronounced in H ($p<0.02$; ANOVA). After losartan administration, 5αR declined only in H ($p<0.02$; ANOVA) and similarly A activity declined in H, but not in D ($p<0.006$; ANOVA).
Conclusion: In type 2 diabetes, lower DHT levels and 5αR activity was found compared to healthy subjects. Acute hyperinsulinaemia declines DHT concentrations; the decrease is more pronounced in H, in D the reaction is mittigated. Losartan administration results in an increase in DHT, also more pronounced in H. AT-1 receptor blockade could thus prevent untoward decline of DHT during acute hyperinsulinaemia.

Serum concentrations of DHT and activity of 5αR in D and H.

		D		K	
		DHT ($nmol.l^{-1}$)	5αR (DHT/T)	DHT ($nmol.l^{-1}$)	5αR (DHT/T)
HIC	0 min.	3.27±0.40	0.284±0.031	2.25±0.34	0.191±0.035
	240 min.	2.45±0.35	0.203±0.031	2.19±0.38	0.151±0.027
AT-HIC	0 min.	3.54±0.64	0.268±0.050	2.59±0.53	0.184±0.049
	240 min.	2.52±0.55	0.201±0.029	2.47±0.65	0.183±0.057

Data are expressed as median±standard error. Evaluated with ANOVA with repeated measures, statistic significance in text.

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0580

Glucocorticoids impair glucose metabolism of isolated rat skeletal muscle via a direct non-genomic mechanism
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Background and Aims: Increased concentrations of circulating glucocorticoids are known to impair glucose homeostasis and are considered to contribute to the development of type 2 diabetes. This has been attributed to the activation of the cytosolic glucocorticoid receptor, which affects pancreatic insulin secretion and visceral fat accumulation. Although skeletal muscle is the most important tissue for insulin-induced glucose lowering, the possible contribution of glucocorticoid effects on muscle has hardly been studied. To close this gap in knowledge, we examined the direct effects of glucocorticoids on glucose handling by skeletal muscle.
Materials and Methods: Freshly isolated specimens of rat soleus muscle were exposed to corticosterone, which is the predominant glucocorticoid in rats and was used at concentrations ranging from 1 μM (corresponding to diurnal peak plasma levels) to 50 μM. During the last hour of incubation, rates of glucose metabolism were measured under stimulation with 25 nM insulin (if not stated otherwise). Pharmacological blockers and other steroids were used to analyse the mechanims underlying the observed effects.
Results: 5h of corticosterone exposure dose-dependently reduced insulin-stimulated glycogen synthesis in the muscle specimens (control: 3.5±0.1 μmol glucose incorporated into $glycogen*g^{-1}*h^{-1}$; changes induced by corticosterone: 1 μM, −0.4±0.2; 10 μM, −0.5±0.2; 50 μM, −1.1±0.2; $p<0.05$ each), which resulted in decreased glycogen content (control: 11.7±0.7 μmol glucosyl units/g; changes induced by corticosterone: 1 μM, −1.4±0.3; 10 μM, −1.6±0.7; 50 μM, −1.7±0.8; $p<0.05$ each). Rates of glucose oxidation were reduced in parallel (control: 1.03±0.04 μmol $glucose*g^{-1}*h^{-1}$; changes induced by corticosterone: 1 μM,+0.10±0.05, ns; 10 μM, −0.15±0.04, $p<0.05$; 50 μM, −0.43±0.07; $p<0.001$). Relative impairment of glycogen synthesis was similar in the presence and absence of various concentrations of insulin and, hence, could not be attributed to blunted insulin sensitivity (% decrease induced by 50 μM corticosterone: no insulin, −34±11%; 0.3 nM insulin, −27±7%; 3 nM insulin, −34±11%; 30 nM insulin, −34±11%; $p<0.02$ each). Furthermore, inhibition of glycogen synthesis by 50 μM corticosterone occurred rapidly (μmol $glucose*g^{-1}*h^{-1}$: after 30 min, 8.0±0.6 vs.

5.7±0.3; after 90 min, 6.1±0.2 vs. 2.3±0.5; p<0.01 each) and was not affected by concomitant exposure to an inhibitor of transcription (5 h, without vs. with 1 mg/l actinomycin D: −1.5±0.2 vs. −1.5±0.4; ns), which indicates a non-genomic mechanism of action. Glycogen synthesis was also reduced by 50 μM 11-dehydro-corticosterone (−1.7±0.2; p<0.001), but was hardly affected by 50 μM dexamethasone (−1.0±0.4, p=0.06, ns), which contrasts with the agonistic activities of these compounds at the cytosyolic glucocorticoid receptor (dexamethasone>-corticosterone>>11-dehydro-corticosterone).
Conclusion: Glucocorticoids directly impair glycogen storage and glucose oxidation in isolated rat skeletal muscle, which is mediated by a non-genomic mechanism of action and does not involve the "classic" cytosolic glucocorticoid receptor. This non-genomic pathway could contribute to glucocorticoid-induced impairment of glucose metabolism *in vivo*.

0581

Effects of dehydroepiandrosterone (DHEA) on AMPK activity and gene expression in fat tissue

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Background and Aims: Numerous evidences indicate DHEA reduce adipose tissue in men and animals, however precise mechanism has been unclear. In this study, we investigated the effects of DHEA treatment on gene expression in adipose tissue. Furthermore, we measured AMP-activated kinase (AMPK) activity, which is the target of leptin and adiponectin, in fat tissue.
Materials and Methods: Epididymal fat was obtained from male C57BL mice after breeding with or without (control) 0.5% DHEA containing food for 7 day. Approximately 30000 genes were analyzed with Acegene Oligo Chip (HitachiSoft, Japan). AMPK activity was determined by the amount of phospho-AMPK (pAMPK) and phospho-acetyl CoA carboxylase (pACC) levels.
Results: DHEA decreased adipose tissue weight by 22%. It elevated p-ACC levels significantly. Three hundred four genes were upregulated more than 200% (vs control) with DHEA administration, whereas 361 genes were reduced less than 50%. Although few genes involved in energy metabolism such as malonyl-CoA decarboxylase were affected, genes involved in intracellular signal transduction, such as Rho GTPase activating protein 23, integrinα7, protein phosphatase 1, ras homolog gene family were increased, and genes of phosphatidylinositol 3-kinase, regulatory subunit, (p85α), ADP ribosylation like 6, rho-associated coiled-coil forming kinase 2 decreased. Many genes regulating transcription and cell cycle, such as foxa1, telometic repeat binding factor 2, interacting protein, retinoblastoma protein. Although apoptosis-related genes, such as caspase 3, were upregulated, no evidences of apoptosis, such as cleaved caspase 3 and cleaved PARP, were detected.
Conclusion: These results suggested that DHEA regulates the amount of adipose tissue through the expression level of genes involved in intracellular signaling and transcription.

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Glucagon hypersecretion underlies glucose intolerance in CAPS-deficient mice

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Background and Aims: CAPS1 (Ca^{2+}-dependent activator protein for secretion) is thought to play an essential role in mediating exocytosis from transmitter storing large dense-core vesicles (LDCVs) in neuroendocrine cells. Antibody inhibition studies in chromaffin cells, melanotrophs and mouse pancreatic beta-cells indicate that CAPS1 may be required for a late release phase of LDCV. However, our studies on perinatal lethal CAPS1-knockout mice (CAPS1 −/− mice) indicated that CAPS1 rather influences the uptake or storage of transmitters in LDCVs than their exocytosis. The second isoform, CAPS2, is encoded by a separate gene, but much less is known about its function. Here we have investigated the roles of CAPS1 and CAPS2 in adult pancreatic alpha- and beta-cells.
Materials and Methods: Animal models used are: Wild-type (WT), single homozygous CAPS2 knockout mice (CAPS2 −/−), and double heterozygous CAPS1 and homozygous CAPS2 knockout mice (CAPS1+/−; CAPS2 −/−). These mouse models have been investigated by immunocytochemistry, single cell capacitance measurements of exocytosis, as well as *in vitro* and *in vivo* islet hormone release.
Results: Immunocytochemistry with CAPS1- or CAPS2-specific antibodies revealed that both CAPS isoforms are widely co-expressed in wild-type islets of Langerhans. Life expectancy and fertility is unaffected in CAPS2 −/− and CAPS1+/−; CAPS2 −/− mice. Glucose tolerance is impaired in CAPS1+/−; CAPS2 −/− mice compared to CAPS2 −/− mice (12.6±0.9 vs 10.3±0.4 mmol/l; p<0.04). Interestingly, *in vivo* and *in vitro* insulin secretion was not different in CAPS1+/−; CAPS2 −/− and CAPS2 −/−

mice. By contrast *in vitro* glucagon release data clearly indicate hypersecretion of glucagon in CAPS1+/−; CAPS2 −/− mice compared to CAPS2 −/− mice at 1 mM glucose (100.4±11.4 vs. 64.3±8.3 pg/islet/h; p<0.001), as well as 20 mM glucose (92.8±11.3 v. 56.8±12.0 pg/islet/h; p<0.03). In addition, overall morphology of the pancreas is affected by CAPS deficiency.

Conclusion: We conclude that a) CAPS1 and CAPS2 protein function is (at least in part) overlapping; b) CAPS deficiency results in glucose intolerance, and c) CAPS deficiency specifically affects the secretory capacity of pancreatic alpha−cells. These effects may be secondary to pancreatic development.

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Acute effects of the CB1 receptor antagonist AVE1625 on metabolic biomarkers in wistar rats

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Background and Aims: In acute preclinical studies CB1 receptor antagonists unequivocally demonstrated a powerful reduction of food consumption and body weight, which was more pronounced during starved-to-refed conditions. In multiple dose studies CB1 receptor antagonists showed sustained reductive effects on body weight, but only a transient anorectic action. This indicates, that (i) the effect of CB1 receptor antagonists must also be present during normal fed conditions, and (ii) that CB1 receptor antagonists should decrease food effectiveness or in other words increase energy expenditure. The objective of the present study was to investigate mechanistically in fed Wistar rats, whether AVE1625 causes primary effects on metabolic blood and tissue parameters as well as energy balance, which are independent of reduced caloric intake.

Materials and Methods: AVE1625 was administered to normal fed male Wistar rats at doses of 10 and 30 mg/kg. Blood was collected 2 h, 5 h and 6 h after compound administration for determination of metabolic blood parameters. After 6 h the rats were killed, the liver was removed for determination of hepatic glycogen content. In the setting of indirect calorimetry the effects of acutely administered 30 mg/kg of AVE1625 on O_2-consumption and CO_2-production was measured; energy expenditure, RQ, the oxidation rates of fat and carbohydrates were calculated.

Results: AVE1625 caused a slight dose-dependent increase in basal lipolysis. In spite of increased free fatty acids there was a tendency for a time-dependent decrease of triglycerides within the study period. 6 h after a single administration of the drug liver glycogen content was dose-dependently reduced to 40–50% compared to that of untreated controls. These findings demonstrate a primary acute effect of AVE1625 on induction of (i) lipolysis from fat tissue (increased FFA) and (ii) glycogenolysis from the liver (reduced hepatic glycogen). Measured by indirect calorimetry, AVE1625 caused an immediate increase in total energy expenditure, a transient increase of glucose oxidation and a long-lasting increase of fat oxidation, which were consistent with the acute findings on metabolic blood and tissue parameters.

Conclusion: We conclude that in addition to the well-investigated effect to reduce body weight by CB1 receptor antagonists due to reduced caloric intake, AVE1625 increased lipid oxidation induced by persistently increased lipolysis from fat tissues, which might significantly contribute to the weight reducing effect of AVE1625. Furthermore, the improvement of serum triglyceride levels appeared to be caused by an additional primary effect of AVE1625 on intermediary metabolism rather than a secondary effect due to reduced body weight.

PS38

Glucose transport

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Insulin and IGF1 effects on glucose transporter expression in monocytes from obese subjects

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Background and Aims: IGF1 exhibits metabolic effects similar to those of insulin. Administration of IGF1 to subjects with insulin resistance reduces hyperglycaemia and insulin requirements, implying that this peptide may compensate for the defect in insulin action. Obesity is associated with insulin resistance and is a major predisposing factor for type 2 diabetes. The aim of our study was to examine the effects of IGF1 and insulin on the translocation of GLUT3 and GLUT4 glucose transporter isoforms on the plasma membrane of monocytes from obese subjects.

Materials and Methods: Blood was withdrawn from 7 non-obese ([NOB], BMI 24+1Kg/m^2, HOMA 1.5+0.5) and 6 obese subjects ([OB], BMI 37+1Kg/m^2, HOMA 3.3+ 0.6). Circulating mononuclear cells from blood were obtained by Histopaque gradient centrifugation. Cells were incubated for 1 hour at various concentrations of insulin (0, 0.07, 0.17, 0.7 nM) and IGF1 (0, 0.07, 0.14, 0.7 nM) to determine the expression of GLUT3 and GLUT4 isoforms on the plasma membrane. After termination of the incubation, monocytes were stained with CD14-PE antibody and anti-human GLUT3 or GLUT4 antisera conjugated with Alexa488 and anti-IgG-Alexa488 as an isotype control. Flow cytometry was performed utilizing a FACS Calibur (Becton-Dickinson). The data were analyzed using Cell Quest software and presented as Mean Fluorescence Intensity (MFI). Statistical analysis of insulin and IGF1 dose-response curves has been done with repeated-measures analysis of variance.
Results: (**A**) *Monocytes from NOB*: Insulin and IGF1 increased plasma membrane GLUT4 and GLUT3 levels in a dose-dependent manner, with a maximal response at 0.7 nM for each hormone. Insulin increased GLUT4 levels by 42% (211+19 and 299+24 MFI at 0 and 0.7 nM respectively, $p<0.0001$) and GLUT3 levels by 39% (302+21 and 419+22 MFI at 0 and 0.7 nM respectively, $p<0.0001$). The respective increases for IGF1 were 21% in GLUT4 (207+27 and 251+34 MFI, $p=0.0005$) and 25% in GLUT3 (308+27 and 384+44 MFI, $p=0.0005$). (**B**) *Monocytes from OB*: Insulin did not increase the levels of GLUT4 on the plasma membrane (197+27 and 216+0.5 MFI, at 0 and 0.7 nM respectively, $p=0.2$) but increased those of GLUT3 (301+9 and 348+7 MFI, at 0 and 0.7 nM respectively, $p=0.0016$); the latter increase was significantly less ($p=0.02$) than that observed in NOB. IGF1 increased both GLUT4 (197+27 and 232+21 MFI, $p=0.0016$) and GLUT3 (298+12 and 354+5 MFI, $p=0.0016$) and these responses were not different from those in NOB.
Conclusion: In obese subjects, translocation of GLUT4 and GLUT3 on the plasma membrane in response to insulin is impaired whilst that in response to IGF1 is retained. The results suggest that although insulin and IGF1 share common signaling pathways, their actions are distinct. This may imply a plausible compensatory role of IGF1 on glucose homeostasis in obesity and insulin resistance.

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Activation of p38 MAP kinase is essential for oxidant stress-induced glucose transport in rat skeletal muscle

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Background and Aims: We have previously demonstrated that an oxidant stress (hydrogen peroxide) can acutely activate the glucose transport system in isolated rat skeletal muscle. In addition, this oxidant stress simultaneously engages the stress-activated protein kinase p38 mitogen-activated protein kinase (p38 MAPK). Previous investigations from other research groups indicate that activation of p38 MAPK can modulate the glucose transport system in insulin-sensitive cells and tissues. However, it is currently unclear whether the activation of p38 MAPK is mechanistically involved in the stimulatory effect of hydrogen peroxide on the mammalian skeletal muscle glucose transport system. Therefore, the purpose of the present study was to assess the role of p38 MAPK activation in oxidant stress-stimulated glucose transport in type IIb (epitrochlearis) and type I (soleus) skeletal muscle of insulin-sensitive lean Zucker rats.
Materials and Methods: Isolated muscles were incubated in 8 mM glucose for 2 hr in the absence or presence of 100 mU/ml glucose oxidase to produce hydrogen peroxide (60–90 μM). In addition, muscles were incubated without or with A304000, a selective inhibitor of p38 MAPK. Glucose transport activity was assessed by 2-deoxyglucose uptake, and phosphorylation of p38 MAPK and Akt was performed using standard immunoblotting methods.
Results: Hydrogen peroxide activated glucose transport by 53–66% ($p<0.05$) in both muscle types, and this oxidant stress increased thr180/tyr182 phosphorylation of p38 MAPK by 40–50%. Exposure to 10 μM A304000 in the presence of hydrogen peroxide had the desired effect of substantially decreasing p38 MAPK phosphorylation without diminishing activation of other kinases associated with glucose transport stimulation, such as Akt ser473 phosphorylation. Importantly, co-treatment with the p38 MAPK inhibitor A304000 essentially abolished the oxidant stress-induced increase in glucose transport in both epitrochlearis and soleus muscles.
Conclusion: Collectively, these data underscore the critical role of p38 MAPK in the acute activation of the glucose transport system by an oxidant stress. The oxidant stress-induced activation of p38 MAPK was essential in both type I and type IIb muscles for stimulation of glucose transport. Further studies are needed to define these interactions between p38 MAPK and the muscle glucose transport system.

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Insulin signaling and glucose transport in skeletal muscle from first-degree relatives of type 2 diabetic patients

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Background and Aims: Aberrant insulin signaling and glucose metabolism in skeletal muscle from type 2 diabetic patients may arise from genetic defects and an altered metabolic milieu. To avoid the influence of hyperglycaemia, we determined insulin action on signal transduction and glucose transport in isolated *vastus lateralis* skeletal muscle from normoglycaemic subjects at high risk for developing type 2 diabetes: first-degree relatives of type 2 diabetic patients.
Materials and Methods: Normal glucose tolerant first-degree relatives of type 2 diabetic patients (n=8, 41±3 yrs, BMI 25.1±0.8 kg/m^2) and healthy control subjects (n=9, 40±2 yrs, BMI 23.4±0.7 kg/m^2) with no family history of diabetes were studied. Smaller muscle strips were prepared from open muscle biopsy specimens, and exposed to different concentrations of insulin. Insulin signaling intermediates were determined by Western Blotting. mRNA of key transcriptional factors and co-regulators of mitochondrial biogenesis were determined by Real-Time PCR.
Results: Basal and sub-maximal insulin-stimulated (0.6 and 1.2 nM) glucose transport was comparable between groups, whereas the maximal response (120 nM) was 38% lower ($P<0.05$) in the relatives. Insulin increased phosphorylation of Akt and its substrate AS160 in a dose-dependent manner, with comparable responses between groups. AS160 phosphorylation and glucose transport were positively correlated in controls ($R^2=0.97$, $P=0.01$), but not in relatives ($R^2=0.46$, $P=0.32$). Skeletal muscle mRNA expression of PGC-1α, PGC-1β, PPARδ, NRF-1 and UCP-3 was comparable between first-degree relatives and control subjects.
Conclusion: The uncoupling of insulin action on Akt/AS160 signaling and glucose transport implicates defective GLUT4 trafficking may constitute an early event in the pathogenesis of type 2 diabetes.
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hSGLT5 (SLC5A10)– a novel human sodium-dependent sugar transporter in the kidney

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Background and Aims: Monosaccharides are absorbed by the small intestine and are reabsorbed in the kidney from primary urine back into the blood. Sodium-dependent sugar transporters are essential to transport the sugars across the plasma membranes. The aim of the present study was to examine the expression and function of a novel member of the human sodium/glucose cotransporter family, the sodium-dependent sugar transporter hSGLT5 (SLC5A10).
Materials and Methods: The localization of the hSGLT5 gene expression in 20 human tissues was determined by the quantitative RT-PCR and compared with the expression pattern of hSGLT2 in these tissues. Concrete localization of hSGLT5 was determined by in-situ hybridization in the kidney.
hSGLT5 cDNA isolated from human kidney RNA was cloned and transfected in HEK293 cells. The function of hSGLT5 was characterized in the FLIPR® membrane potential assay by detecting the Na^+-dependent transport activity of hSGLT5 in the presence of different sugars in the transfected cells. Furthermore [^{14}C]AMG (α-methyl-D-glucose) and [^{14}C]galactose uptake into the HEK/hSGLT5 cells was measured. Monosaccharides and SGLT-inhibitors were added to examine their inhibitory activity towards the [^{14}C]AMG uptake.
Results: It could be shown by quantitative expression analysis that hSGLT5 is selectively expressed in the kidney. The expression pattern corresponds to that of hSGLT2 which is essential for the reabsorption of glucose in the kidney.
The in-situ hybridization revealed the coexpression of hSGLT2 and hSGLT5 mainly in epithelial tissues of the glomerulus and distal and proximal tubulus, a low expression could be shown for the collecting ducts.
In an membrane potential assay the monosaccharides galactose, glucose and fructose lead to a Na^+-dependent membrane depolarization whereas AMG and mannose only showed a smaller effect.
The examination of the [^{14}C]AMG uptake into HEK/hSGLT5 cells revealed a Na^+-dependent transport of [^{14}C]AMG into the cells. This transport could be inhibited by hexoses, galactose being the most potent one. It could also be shown that hSGLT5 actively transports [^{14}C]galactose. Furthermore the [^{14}C]AMG and [^{14}C]galactose uptake could be inhibited by Phlorizin and T1095A in a concentration dependent manner.
Conclusion: These results suggest that hSGLT5 is a sodium-dependent sugar transporter, which is mainly expressed in the kidney and serves for the reabsorption of different hexoses.

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Impaired glucose uptake and overexpression of glucotransporter 1 (GLUT1) and glucotransporter 3 (GLUT3) are characteristic of natural killer (NK) cells from subjects in the prediabetic state

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Background and Aims: Diabetic hyperglycemia is associated with impaired cellular glucose transport. Moreover, fluctuations in blood glucose levels are known to affect peripheral blood cells. This relation can be particularly expressed by glucose transporter proteins and glucose uptake by cells of the peripheral blood. Studies of the glucose transport activity of NK cells in the prediabetic state may contribute to the better understanding of the pathogenetic phenomenon in the natural history of Type 2 diabetes. The earlier results revealed significant differences in glucose transport between NK cells of healthy subjects and Type 2 diabetic patients. Therefore the aim of this study was to determine the quantitative expression of glucotransporters GLUT1 and GLUT3 and to evaluate potential abnormalities of glucose uptake in NK cells obtained from prediabetic persons in comparison with healthy subjects.
Materials and Methods: The study group included 10 patients with a diagnosis of the prediabetic state, naive to any hypoglycaemic drugs. As a control group 9 carefully matched healthy subjects were enrolled. Lymphocytes of the peripheral blood were obtained by Ficoll-Isopaque gradient centifugation. NK cells were isolated and removed by anti-CD16 antibody treatment. Glucose transport was monitored with deoxy-D-glucose (2-[^{3}H(G)]). At previously assigned time points (15, 30, 60 minutes of incubation) deoxy-D-glucose uptake was stopped and its concentration in the cells was measured by scintillation counting. The expression of GLUT1 and GLUT3 was investigated by flow cytometry, which was performed utilizing a FACSCalibur (Becton-Dickinson). Indirect immunofluorescence was applied as a staining technique. Cells were stained by using anti-human GLUT1,3 antibodies (R&D Systems) and FITC-conjugated immunoglobulin. The data was analyzed using Cell Quest software and presented as a percentage of cells revealing expression of the determined receptor protein.
Results: The study revealed the significant decrease of deoxy-D-glucose transport in NK cells obtained from prediabetic subjects ($P<0.001$). The mean values of 4260 ccpm at 15 min. 8538 ccpm at 30 min. and 10925 ccpm at 60 min. were obtained in NK cells of healthy subjects. The glucose uptake was, respectively, 2076 ccpm, 4151 ccpm and 7907 ccpm in NK cells of prediabetic persons. The decrease of glucose uptake correlated with the higher levels of GLUT1 and GLUT3 in the studied cells. Flow cytometry showed the substantial increase in GLUT1 and GLUT3 proteins level in NK cells of prediabetic patients (43±3,7% and 55±4,3% respectively) in comparison with the expression of 21±1,9%-GLUT1 and 24±2,3%-GLUT3 in NK cells of healthy subjects.
Conclusion: Cellular glucose transport in NK cells is significantly impaired in the prediabetic state. The high expression of GLUT1 and GLUT3 in NK cells of prediabetic patients is associated with a different pattern of glucose transport in these cells. Overexpression of glucotransporters may become a significant factor in evaluation of glucose metabolic disturbances induced by insulin deficiency in type 2 diabetes. Alterations of GLUT proteins expression in NK-cells seem to be an interesting model system which may be used in recognition of the prediabetic state and evaluation of preventive actions.

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Effects of the SGLT2-inhibitor AVE2268 on urinary glucose excretion (UGE) and blood glucose in mice, rats and dogs

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Background and Aims: Na^+ -dependent glucose transporters (SGLT's) exist in at least two forms. The SGLT1 is present in the brush border membrane of the intestine and transports glucose into the enterocytes. Both, SGLT1 and SGLT2 are found in the kidney and transport glucose from the primary urine into the tubular cells. Inhibition of glucose re-absorption in the kidney by phlorizin has been known for a long time. In diabetic rodents phlorizin have beneficial effect in experimental type 2 diabetes. However, phlorizin is not absorbed from the gut. The search for oral active inhibitors of SGLT2 led to the development of AVE2268, a substituted glucopyranosid. In vitro studies on isolated brush border membrane vesicles showed that AVE2268 inhibited SGLT2 with an IC_{50} of 6 μM, the affinity to SGLT1 was lower (IC_{50}=36 μM). The aim of the study was, to validate the proof of principle (SGLT2-activity) of AVE2268.
Materials and Methods: Dose and time-dependency of urinary glucose excretion (UGE) was tested in mice, rats and dogs. The effect of AVE2268 on blood glucose after a glucose load was tested in mice and rats. Postprandial blood

glucose was measured in Zucker diabetic fatty (ZDF) rats after a meal.
Results: AVE2268 stimulated UGE in a dose-dependent fashion in mice, rats and dogs. Mice were the most sensitive species, rats being 5-fold less sensitive. In the time-course experiment in rats, UGE increased from the 1st h and peaked after 6 to 8 h. AVE2268 decreased blood glucose in mice and rats in a dose-dependent fashion, following an oral or intraperitoneal glucose tolerance test. Postprandial blood glucose was dose-dependently lowered in ZDF rats after a meal.
Conclusion: The present experiments clearly demonstrate that AVE2268 is a preferential inhibitor of SGLT2 in the kidney. Blood glucose after a glucose challenge was lowered in healthy and diabetic rodents. AVE2268 is a new antidiabetic drug that may represent an innovative approach for the treatment of type 2 diabetes.

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Loss of detection of sugar abundance in GLUT2 expressing tissues deeply alters mouse glucose homeostasis

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Background and Aims: We are investigating the mechanisms by which cells sense nutrients and adapt to their nutritional environment. Glucose is not only a substrate for most mammalian cells but it also generates a signal to the nucleus that regulates gene transcription. In hepatic cells, we can block the stimulation of glucose sensitive gene transcription by inhibiting metabolism or by expressing a GLUT2 loop domain that leaves unaffected glucose metabolism. Our aim was to evaluate *in vivo*, the importance of the detection of sugar abundance triggered by GLUT2
Materials and Methods: We took advantage of the tool that we developed *in vitro*, to produce transgenic mice that expressed the GLUT2 loop domain driven in most tissues by an actin promoter. We expected thus to modulate the detection of sugar in GLUT2 expressing tissues i.e. liver, kidney, pancreas, intestine and brain. We measured the perturbations induced by the transgene on glucose homeostasis and on sugar-sensitive gene expression.
Results: Transgenic mice presented growth retardation, which was correlated to transgene expression levels. This occurred despite normal daily food intake and blood glucose concentrations. In response to an oral glucose challenge, we observed pronounced glucose intolerance. Moreover, during OGTT, significantly reduced plasma insulin concentrations and 2 fold increased glycosuria underlined defects in pancreas and kidney functions. Nevertheless, insulin tolerance tests were unchanged suggesting that peripheral tissues that are not expressing GLUT2, were unaffected by the transgene. By indirect calorimetry, we recorded that mice favored lipid over glucose oxidation that was delayed by 3 hours after a mixed meal. A poor central control of satiety in transgenic mice is suspected because meal consumption was not reduced appropriately after a glucose injection. Importantly, in liver, stimulation of gene expressions by sugar diet was almost abolished, but their basal levels were unchanged. These transgenic mice appeared thus unable to detect sugar abundance, but kept the functions displayed by GLUT2 expressing tissues at basal levels. The molecular role of GLUT2 loop domain might be either to anchor proteins of sugar signaling pathway or to mediate transcription activation.
Conclusion: Taken together, these data suggest that the detection of sugar abundance mediated by GLUT2 in liver pancreas, kidney and brain, controls multiple aspects of glucose homeostasis. Perturbations of this physiological process might participate to metabolic diseases.
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Regulating cell surface GLUT4 arrival and fusion in L6 skeletal muscle cells

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Background and Aims: Skeletal muscle and adipose are the main insulin-responsive tissues to facilitate glucose uptake via the GLUT4 glucose transporter. Recently, phosphorylation of an Akt substrate and putative RabGAP, AS160, was implicated in GLUT4 traffic in cultured 3T3-L1 adipocytes. Insulin-stimulated AS160 phosphorylation was also shown to be impaired in skeletal muscle of type 2 diabetic patients. However, little is known of the mechanism(s) by which AS160 regulates GLUT4 traffic in skeletal muscle. We have recently shown that GLUT4 arrival and/or fusion leading to glucose uptake can be differentially modulated by insulin signaling molecules and phospholipids. Thus, our aim in the present study was to determine whether AS160 regulates GLUT4 arrival and/or fusion in L6 skeletal muscle cells.
Materials and Methods: To this end, we explored the effects of transient expression of wild type (WT) or a non-phosphorylatable mutant (4P) of AS160 using an L6 skeletal muscle cell line stably expressing GLUT4myc.

By transient transfection of cDNA encoding tetanus toxin light chain (TeTx), which cleaves the toxin-sensitive VAMP2, we also examined its role in mediating surface GLUT4 arrival or fusion. Immunostaining intact or permeabilized "rounded" L6 cells with antibodies directed to the transporter's cytosolic C-terminus or exofacial myc epitope respectively allows detection of GLUT4 arrival or fusion at the plasma membrane.

Results: We show transient expression of AS160 4P prevents insulin-stimulated plasma membrane fusion but not arrival of GLUT4myc. WT AS160 over-expression slightly inhibits insulin-mediated surface GLUT4myc fusion, but not its arrival. Similarly, cleavage of VAMP2 by TeTx prevents GLUT4myc fusion without perturbing cell surface transporter arrival. In contrast, cytoskeletal disruption by cytochalasin D diminishes largely insulin-induced plasma membrane GLUT4myc fusion and partially arrival. Interestingly, AS160 4P also appears to 'cluster' the intracellular GLUT4 in the perinucleus.

Conclusion: Altogether, these data suggest AS160 is an unlikely RabGAP for modulating GLUT4 vesicle movement along cytoskeletal tracks, which partially facilitate insulin-stimulated transporter arrival to the cell surface. We propose that AS160 may regulate the plasma membrane-associated docking and/or fusion of GLUT4 vesicles. It will be interesting to note which Rabs are required for these AS160-dependent events. VAMP2 has a major role in fusion of GLUT4 rather than arrival of GLUT4 at the cell periphery.

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Atorvastatin treatment inhibits differentiation and glucose transporter 4 expression in 3T3-L1 adipocytes and impairs glucose tolerance in diabetic NSY mice

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Background and Aims: HMG-CoA reductase inhibitors (statins) are widely used for controlling hypercholesterolemia and hyperlipidemia, which often occur in patients with type 2 diabetes mellitus. Therefore, type 2 diabetic patients are often treated with statins. However, disadvantageous influence of statins on glucose metabolism has also been reported. Adipocytes have been shown play an important role in glucose metabolism. This study aimed to explore possible effects of statins on the differentiation and glucose handling in adipocytes.

Materials and Methods: Effects of atorvastatin, simvastatin and pravastatin on 3T3-L1 cells in the period during differentiation (culture days 1–7) and on those after differentiation (culture days 8–10) were examined. Adipocyte maturation was determined morphologically. Protein levels and mRNA expressions of glucose transporter 4 (GLUT4) and adipocyte marker proteins were determined by immunoblotting and real-time RT-PCR, respectively. In type 2 diabetic NSY mice, following treatment with atorvastatin for 15 weeks, glucose and insulin tolerance tests were performed and GLUT4 levels in white adipose tissue were examined.

Results: Treatment with atorvastatin (1 ng/ml) inhibited adipocyte maturation, reduced protein levels and mRNA expressions of GLUT4 and C/EBPα, and inhibited insulin-induced glucose uptake in 3T3-L1 cells. In differentiated 3T3-L1 adipocytes, atorvastatin also reduced, though to a lesser extent, GLUT4 and C/EBPα protein levels and mRNA expressions and insulin-induced glucose uptake. These effects were reversed by L-mevalonate or geranylgeranylpyrophosphate. Neither simvastatin nor pravastatin even at higher doses exerted these effects. In NSY mice, atorvastatin accelerated glucose intolerance due to insulin resistance and decreased GLUT4 in white adipose tissue.

Conclusion: Atorvastatin attenuates adipocyte differentiation and GLUT4 expression by inhibiting isoprenoid biosynthesis and impairs glucose tolerance in type 2 diabetic NSY mice.

PS 39

Insulin action - human studies

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The study on insulin secretion and insulin action in obese Chinese diabetic patients with unprovoked ketosis

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Background and Aims: The frequency of newly diagnosed obese diabetic patients with unprovoked ketosis was increased in recent years in China. The mechanisms of unprovoked ketosis in these subjects were not quite clear. The aim of the present study was to evaluate the peripheral insulin sensitivity and pancreatic β cell insulin secretion function in obese Chinese diabetic patients with unprovoked ketosis, and to explore the possible mechanism of unprovoked ketosis in obese diabetic subjects.

Materials and Methods: Two groups of newly diagnosed diabetic patients were involved in this study: OB-DK group: 10 obese diabetic patients with unprovoked ketosis;

T2DM group: 10 non-obese, non-ketosis type 2 diabetic patients. 10 healthy lean volunteers (C) and 10 obese persons with normal glucose tolerance (OB) were enrolled as control. The ICA, IAA, and GAD-Ab were negative in all subjects. The serum insulin, C-peptide, free fatty acids (FFAs) levels were measured at baseline. Then subjects underwent a euglycemic-hyperinsulinemic clamp study (insulin infusion rate: 120 mU/min/kg), intravenous glucose tolerance test (IVGTT) and hyperglycemic clamp study to evaluate the insulin action and insulin secretion.

Results: In OB-DK group, the area under curve ($AUC_{10\ min}$) was smallest (C: 352.9±125.8 mU/L,OB: 672.6±262.1 mU/L,T2DM: 187.2±75.8 mU/L,OB-DK: 79.9±28.4 mU/L, $p<0.05$ when compared with C, OB, T2DM). The second-phase insulin response and glucose infusion rate during steady state of hyperglycemiac clamp were lower in OB-DK when compared with T2DM (2nd Ins: 10.8±3.7 mU/L, vs. 44.7±18.8 mU/L, $p<0.05$; GIR: 1.14±0.57 mg/Kg.min vs. 7.62±2.57 mg/Kg.min, $p<0.05$). The glucose disposal rates during steady state of euglycemic- hyperinsulinemic clamp were very low in DK-OB group (C: 12.8±1.5 mg/kg.min, OB: 6.1±1.2 mg/kg.min, T2DM: 9.7±1.1 mg/kg.min; DK-OB: 4.3±1.6 mg/kg.min). The serum FFAs levels were highest in OB-DK group (C: 364.3±158.6 μmol/L, OB: 887.9±157.9 μmol/L, T2DM: 603.4±289.1 μmol/L, OB-DK: 1019.4±372.8 μmol/L, $p<0.05$ when compared with C, OB, T2DM).

Conclusion: Severely peripheral insulin resistance and markedly impaired β-cell insulin secretion were observed in obese diabetic patients with unprovoked ketosis. The potential mechanism of ketone over-production in newly diagnosed obese diabetic patients may associate with glucolipotoxicity which severely damaged the B-cell function. It is logical to expect that these diabetic patients may belong to type 2 diabetes.

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0594

Differences in insulin signaling kinetics and glucose transport of human visceral and subcutaneous adipocytes differentiated *in vitro* from precursor stromal cells

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Background and Aims: visceral fat accumulation is a strong determinant of the metabolic and cardiovascular abnormalities that develop in the metabolic syndrome. The objective of this study was to identify signaling and biological differences in adipocytes differentiated *in vitro* from precursor stromal cells isolated from abdominal subcutaneous (SC) and visceral omental (O) fat biopsies.

Materials and Methods: preadipocytes were obtained from paired SC and O biopsies from 6 non-obese subjects with normal insulin sensitivity, and were differentiated into mature adipocytes *in vitro*. Phosphorylation levels of multiple insulin signaling intermediates were analyzed under basal conditions and after insulin stimulation (10 nM).

Results: Akt phosphorylation on Ser473 and Thr308 peaked at 6 min and tended to decrease at later time points in O adipocytes, whereas a more progressive activation of Akt by insulin was evident in SC adipocytes. No difference was found in Akt protein content in the two fat cell populations. Similarly, GSK-3 phosphorylation by insulin was characterised by a 6-min peak in O adipocytes, which tended to decrease at 30 min, whereas a more persistent phosphorylation was observed in SC cells. Furthermore, in O adipocytes, insulin stimulation induced a rapid increase in Erk-1/2 phosphorylation at 6 min, which returned to basal levels at 30 min. By contrast, insulin did not induce any significant Erk-1/2 phosphorylation in SC adipocytes. Protein content of both Erk-1 and Erk-2 was found to be increased in O compared to SC adipocytes. To verify whether the observed signaling differences translate into changes in biological endpoints, we assessed 2-deoxy-D-glucose uptake in the two fat cell types. Importantly, O adipocytes showed approximately 2-fold higher glucose transport rates compared to SC adipocytes, both in the basal state and following insulin stimulation.

Conclusion: in conclusion, O and SC adipocytes, even when differentiated *in vitro* from precursor stromal cells, display intrinsic differences in the kinetics of insulin signaling activation. Greater glucose transport responses in O adipocytes may be explained by more prominent Akt activation by insulin in this fat cell population.

0595

Insulin sensitivity in anorexia nervosa

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Background and Aims: In anorexia nervosa, the correct measure of insulin sensitivity could constitute a precise marker of the metabolic state, however, due to the different

methodologies utilized in literature, results are not concordant. Our aim was to obtain a method to estimate the variations of insulin sensitivity in anorexic patients by comparing four different indices of insulin sensitivity compared with the gold standard euglycemic hyperinsulinemic clamp.

Materials and Methods: Twelve patients affected by anorexia nervosa (BMI 12.1±1.7 kg/m2) aged 24.8± 2.12 yrs. and 12 control subjects matched for age but with normal BMI (18–25 kg/m2), underwent OGTT and dosage of FT3, FSH, LH, estradiol and cortisol. With the obtained results we calculated insulin sensitivity indexes according to HOMA-R, McAuley, Matsuda and the insulinogenic index.

Results: Although the patients were anorexic, all the surrogate indexes for insulin sensitivity suggested the presence of an increase of this parameter: HOMA-R 0.66 vs. 1.07, p<0,05¸ Matsuda 15.02 vs. 7.35, p<0.002; McAuley 3.05 vs. 1.79 p<0.016. On the contrary, the sensitivity measured by the glucose clamp demonstrated the presence of insulin-resistance M: 5.35 vs 7.16 mg/kg/min p<0.02, even though with a wide range of results. None of the surrogate indexes correlated with the clinical parameters of the disease (FT3; LH; Cortisol, Estradiol). On the contrary, within the group of the anorexic patients, we found a negative correlation between M and FT3 and Estradiol (clamp-FT3: −0.93; clamp–Estradiol: −0.76).

Conclusion: In anorexia nervosa, surrogate indexes usually derived from OGTT or fasting values are not reliable, probably due to the absorptive and secretive alterations present in these patients. The euglicemic–hyperinsulinemic clamp, although difficult to perform, especially in these patients, remains the only reliable method and not replaceable at the moment. The correlation between insulin-resistance and the indexes of remission of the disease seem to suggest (possibly due to remission of the disease and subsequent accumulation of fat in adipocytes) a possible increase of adipokines and may contribute to the difficulty of these patients to recover a normal weight.

0596

Insulin action in adipose tissue and skeletal muscle in hypothyroid patients

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Background and Aims: Although insulin resistance in hyperthyroidism is well documented, information on insulin action in muscle (M) and adipose tissue (AD) in hypothyroidism is limited. This study was undertaken in patients with hypothyroidism to investigate insulin action on glucose disposal and lipolysis.

Materials and Methods: A mixed meal was given to 11 hypothyroid (HO) (age 45+3 yrs, BMI 24+1 kg/m^2, T4 1.6+0.4 μU/dl, TSH 72+10 μU/ml) and 10 euthyroid (EU) subjects (age 42+4 yrs, BMI 24+1 kg/m^2, T4 9+1 μU/dl, TSH 1+0.08 μU/ml). Plasma samples for measurements of glucose (G), insulin (I), triglycerides, glycerol (GLY) and non-esterified fatty acids (NEFA) were taken for 360 min from veins (V) draining the anterior abdominal subcutaneous AD depot and forearm M and from the radial artery (A). Blood flow (BF) in AD and M was measured with ^{133}Xe and strain-gauge plethysmography respectively. *Calculations*: Glucose uptake (GU)=[$G_{(A-V)}$]x[BF]; Fractional glucose uptake (FGU)=$G_{(A-V)}/G_A$; LPL=[$TAG_{(A-V)}$] x[BF]; AD lipolysis=[$GLY_{(V-A)}$x(BF)]–[LPL].

Results: Postprandial changes (area under curve 0–360 min) in HO vs EU: (**1**) Arterial G was not different (2295+159 vs 2063+36 mMmin), but I was increased (15+2 vs 10+0.7U/Lmin, p<0.05). (**2**) BF (1290+79 vs 1579+ 106 ml/100 ml tissue in M and 706+105 vs 1340+144 ml/100 ml tissue in AD) and GU (464+74 vs 850+155 μmol/100 ml tissue in M and 208+42 vs 406+47 μmol/100 ml tissue in AD were decreased (p<0.05), but FGU was not impaired (28+6 vs 33 +6% per min in M and 17+4 vs 14+ 3% per min in AD). (**3**) Suppression of lipolysis by insulin was similar in both groups. (**4**) Arterial triglyceride levels were increased (489 +91 vs 264+36 nmol/Lmin, p<0.05); LPL was similar in both groups (42+11 vs 80+21 μmol/100 ml tissue in AD and 48+10 vs 42+9 μmol/100 ml tissue in M).

Conclusion: In hypothyroidism: (**1**) Glucose uptake in muscle and adipose tissue is resistant to insulin; this may be due, at least in part, to the lower blood flow rates in these tissues. (**2**) Suppression of lipolysis by insulin is not impaired. (**3**) Hypertriglyceridaemia is not due to decreased triglyceride clearance by adipose tissue or muscle.

0597

Effect of insulin on serum CD40L in subjects with normal or impaired glucose tolerance

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Background and Aims: Inflammation is an important pathogenic factor in atherosclerosis and coronary heart

disease, particularly in the context of diabetes, obesity and the metabolic syndrome. The pro-inflammatory mediator, soluble CD40 ligand (sCD40L), which interacts with CD40L expressed on vascular cells, plays an important role in the cascade of inflammatory and proatherothrombotic functions. High serum sCD40L levels have been reported in patients with type 2 diabetes, hypertension, and unstable angina; moreover, its levels increase immediately following an acute myocardial infarction. No information is available on the effects of acute glucose and insulin changes on this marker of atherosclerosis. Aims of the present study were to assess the acute effect of glucose and insulin on sCD40L levels and their relationship with insulin sensitivity in subjects with different degrees of glucose tolerance.

Materials and Methods: 24 subjects with normal glucose tolerance (NGT) and 18 with impaired glucose tolerance (IGT) underwent a 3-h OGTT with measurement of serum sCD40L levels at time 0, 90 and 180 min. Insulin sensitivity was assessed by the Oral Glucose Sensitivity Index (OGIS). To distinguish the role of glucose and insulin, 10 other NGT subjects underwent a 2-h euglycaemic hyperinsulinaemic clamp (insulin infusion: 40 $mU \cdot m^{-2} \cdot min^{-1}$), while a 3-h time-control study (TC) was performed in 10 healthy volunteers.

Results: In comparison with NGT subjects, IGT subjects were heavier (BMI=35.9±2.2 *vs* 28.5±1.5 $kg \cdot m^{-2}$, p=0.006), more insulin resistant (319±12 *vs* 374±12 $ml \cdot min^{-1} \cdot m^{-2}$, p=0.003) and more hyperinsulinaemic both at baseline (122±19 *vs* 86±16 $pmol \cdot ml^{-1}$, p=0.05) and during the OGTT (AUC: 102±11 *vs* 69±8 $nmol \cdot ml^{-1} \cdot 180$ min, p=0.02). Fasting sCD40L was 6.64±0.68 $ng \cdot ml^{-1}$ in NGT and 3.00±0.73 $ng \cdot ml^{-1}$ in IGT (p<0.001 *vs* NGT). A significant reduction of fasting sCD40L concentrations was observed during the OGTT in NGT subjects (from 6.64±0.68 to 2.48±0.50 $ng \cdot ml^{-1}$, p<0.0001) but not in IGT (3.00±0.73 to 2.58±0.88 $ng \cdot ml^{-1}$, p=ns). This reduction was not due to time-related changes since sCD40L levels did not change during the TC study. During the clamp, insulin induced a marked drop in sCD40L levels (from 10.26±2.01 to 1.81±0.58 $ng \cdot ml^{-1}$, p<0.0001). In the pooled data from all studies, fasting sCD40L was reciprocally related to the area under the OGTT glucose curve ($r=-0.32$, p=0.04) and the incremental area under the insulin curve ($r=-0.31$, p=0.05), and directly related to insulin sensitivity ($r=0.30$, p=0.05).

Conclusion: Fasting circulating sCD40L levels are reduced in IGT. Acute hyperinsulinaemia, whether induced by oral glucose administration or by euglycaemic insulin infusion, reduces sCD40L levels only in NGT. The lack of such response in IGT may be related to their insulin resistance and/or to the chronic hyperinsulinaemia.

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0598

Possible polycystic ovary syndrome: Any difference from normal needs to be considered?

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Background and Aims: Recently, the term of 'possible' polycystic ovary syndrome (PCOS) has been used for defining cases whose biochemical evaluations are incomplete, but clinical phenotypes are suggestive of PCOS. In this study, by evaluating medical records of the cases admitted with complaints and symptoms suggestive of PCOS, we aimed to detect the rate of possible PCOS and compare their characteristics and insulin sensitivity status with confirmed PCOS cases.

Materials and Methods: Medical records of 118 women, admitted with complaints and symptoms of PCOS were evaluated retrospectively. Menstrual regularity, hirsutismus, age and body mass index (BMI) on admission were recorded. Insulin sensitivity status of all cases were calculated by Homeostasis Model Assesment of Insulin Resistance (HOMA–IR). Hirsutismus was defined if Modified Ferriman–Gallwey score was found to be over 8 by the same three physicians. Diagnosis of PCOS was performed according to Rotterdam 2003 criteria. Considering medical data regarding complaints of patients and clinical evaluations of physicians on admission, cases were subgroupped as: **a.** Cases with no hirsutism and menstrual dysfunction. (They were evaluated depending on their persistent statements reporting change in body hair.). **b.** Cases with hirsutism only. **c.** Cases with menstrual dysfunction only. **d.** Cases with both hirsutism and menstrual dysfunction. Cases fulfilling Rotterdam 2003 criteria were defined as confirmed PCOS and indeterminate subjects as possible PCOS.

Results: Confirmed PCOS was detected in 70 (59.3%), possible PCOS in 48 (40.7%) cases. Confirmed PCOS was most prevalent in **group d**; confirmed PCOS vs possible PCOS; 4 cases (28.6%) vs 10 (71.4%) in **group a**, 8 (34.8%) vs 15 (65.2%) in **group b**, 26 (63.4%) vs 15 (36.6%) in **group c**, 32 (80.0%) vs 8 (20%) in **group d**, (p=0.000). Body mass index and HOMA–IR values did not differ between groups, confirmed PCOS vs possible PCOS; 25.46±5.55 kg/m^2 vs 26.75±7.55 kg/m^2, 3.37±4.12 vs 3.21±2.50, (p>0.05), subjects with possible PCOS were older (22.04±5.28 years vs 24.87±6.56, p=0.011). Thirty-six (51.4%) cases in confirmed PCOS group and 27

(56.3%) in possible PCOS group had family story of type 2 diabetes mellitus (p>0.05).
Conclusion: Possible PCOS group exhibited similar insulin sensitivity status, family story of diabetes and BMI values with confirmed PCOS group. Considering data demonstrating the role of insulin resistance in PCOS and predominance of pancreatic β-cell dysfunction in PCOS cases who have type 2 diabetic first-degree relatives, we propose that possible PCOS patients may belong to confirmed PCOS group. High rates of confirmed PCOS in **group d** obviously underlines the compliance of patients to medical care as clinical signs become more severe. Their younger age may be explained with their earlier attempts for medical search with full-blown symptoms. Detection of confirmed PCOS in **group a** points at the importance of affected individuals' capability of self-assesment for diagnosis. Changes in body hair recognized only by the patient may better be regarded as serious, even though physical examination does not support. Many PCOS patients seem to be undiagnosed due to inadherence to diagnostic work-up. In addition to Rotterdam 2003 criteria, recognition of any difference from normal needs to be considered.

0599

Different relationship between insulin resistance and body fat in young type 2 diabetic patients of Pakistani and Norwegian ethnicity

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Background and Aims: Immigrants from South Asia to Western countries have a high prevalence of T2DM. We wanted to study the relationship between insulin sensitivity and BMI, abdominal visceral (VAT) and subcutaneous (SAT) adipose tissue, and fatty infiltration in liver, in young patients with T2DM from Norway and Pakistan living in Oslo.
Materials and Methods: Fifteen patients of Pakistani and 15 of Norwegian ethnicity, with mean±SD age 40.3±3.7 and 37.3±6.3, were studied with anthropometric measurements, fasting blood samples, abdominal CT scans and 2 step (40 and 400 mU/m^2/min) euglycemic, hyperinsulinemic clamp, measuring the glucose infusion rate (GIR) in μmol/m^2/min.
Results: Mean BMI, WHR and fasting pl-glucose were similar in the two groups, but the Pakistani group had a significantly higher HbA1c (8.9±1.8 vs 7.3±1.1, p=0.007). A tendency towards lower GIR during clamp was observed in the Pakistani group (320±274 vs 726±743, p=0.057). A significant negative correlation was observed between GIR and BMI in the Norwegian group (r=−0.68, p=0.006), but not in the Pakistani group (r=−0.43, p=0.114). No differences were observed between the groups in the amount of VAT, SAT, or fatty infiltration in liver, measured by Liver/Spleen (L/S) ratio. In the Pakistani group, a significant negative correlation was observed between GIR and VAT (r=−0.76, p=0.001), and a significant positive correlation between GIR and L/S ratio (r=0.64, p=0.01). There was no significant correlation between GIR and VAT (r=−0.39, p=0.15), and GIR and L/S ratio (r=0.43, p=0.13) in the Norwegian group.
Conclusion: All patients of Pakistani ethnicity were insulin resistant, and strong associations were observed between insulin resistance and measures of visceral fat. Patients of Norwegian ethnicity showed larger heterogeneity with respect to insulin resistance.

0600

A 50% overweight reduction using a very low calorie diet, significantly improves insulin-stimulated glucose disposal and skeletal muscle insulin signaling in obese type 2 diabetic patients

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Background and Aims: Energy restriction per sé and weight loss improve blood glucose levels in obese insulin-treated type 2 diabetic patients. Short-term energy restriction decreases basal endogenous glucose production (EGP) with no effect on insulin sensitivity. The blood-glucose lowering mechanism of substantial weight loss has not been fully elucidated in these patients. Especially the effect of weight loss on the disturbed insulin-signaling pathway, leading to impaired translocation of GLUT-4 to the cell membrane, is unknown. The aim of this study was to investigate the effect of considerable weight loss on glucose metabolism, both at the whole-body and at the myocellular level, in obese insulin-treated type 2 diabetic patients.
Materials and Methods: 10 obese (BMI 40.2±1.6 kg/m2 [mean±SEM]) insulin-treated (mean 94±14 U/day) type 2 diabetic patients (HbA1c 7.7±0.4%, FPG 11.1±0.8 mmol/L) were studied during a very low calorie diet (VLCD,

450 kCal/day) on day 2 and again after losing 50% of their overweight (50% OWR). Oral blood glucose-lowering agents and insulin were discontinued 3 weeks prior to and at the start of the VLCD, respectively. EGP and whole-body glucose disposal ([6,6-$^2H_2$]-glucose), lipolysis ([$^2H_5$]-glycerol) and substrate oxidation rates were measured on both study days in basal and hyperinsulinaemic euglycaemic conditions. Skeletal muscle biopsies were obtained from the vastus lateralis muscle in the basal situation and 30 min after the start of the insulin infusion for determination of insulin signaling, insulin-mediated expression of GLUT-4 and FAT/CD36 at the sarcolemma and intramyocellular triglyceride content.

Results: Weight reduction (−20.3±2.2 kg from day 2 to day 50% OWR) normalised basal EGP (20.0±0.9 to16.4±1.2 μmol.kgfatfreemass[FFM]$^{-1}$.min^{-1}) and improved insulin sensitivity, especially insulin-stimulated glucose disposal (18.8±2.0 to 39.1±2.8 μmol.kgFFM^{-1}. min-1, p=0.001). Insulin-stimulated phosphatidylinositol 3′-kinase (PI3K)-activity over basal was significantly higher after weight loss and the down-stream PKB/Akt substrates AS160 and PRAS40 showed absolute increases, both in the basal and the insulin-stimulated situation. Insulin-mediated GLUT-4 content at the sarcolemma was increased in 7 out of 10 patients. Intramyocellular triglyceride content decreased, without an alteration of sarcolemmal FAT/CD36 content. Time required to lose 50% overweight was negatively correlated with the amount of type I fibres at baseline and there was a tendency for an increased percentage type 1 muscle fibres after weight loss.

Conclusion: Considerable weight loss in obese type 2 diabetic patients improves basal EGP and insulin sensitivity, especially insulin-stimulated glucose disposal. This is associated with improved insulin signaling at the level of PI3K, AS160 and PRAS40. The observed decrease in intramyocellular triglyceride content might have contributed to this effect.

PS 40

Insulin signaling

0601

Impaired insulin action despite up-regulation of proximal insulin signaling: novel insights into skeletal muscle insulin resistance in liver cirrhosis

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Background and Aims: Disturbance in glucose metabolism is a common feature in diseases of the liver. This is reflected by a high prevalence of glucose intolerance among patients with hepatic cirrhosis. Hyperinsulinemic euglycemic clamps studies of cirrhotic patients have shown that insulin resistance in skeletal muscles is a major component of the impaired glucose tolerance but the underlying molecular mechanisms are unclear. Treatment with the somatostatin-analogue octreotide suggests that the insulin resistance may be due to inadequate clearance of insulin by the liver, a pathogenesis that is unlike other common forms of insulin resistance. To further characterize skeletal muscle insulin resistance associated with liver disease we examined isolated muscles from an animal model of liver cirrhosis.

Materials and Methods: Cirrhosis was induced by obstruction of the Common Bile Duct. Clinical findings, elevated plasma levels of liver enzymes and histological examinations confirmed cirrhosis. Whole body insulin sensitivity was assessed with an oral glucose tolerance test. Insulin stimulated glucose transport and insulin signaling was measured in isolated muscles incubated *in vitro*.

Results: Cirrhotic animals were insulin resistant on a whole body level and this was at least partly explained by reduced insulin stimulated glucose transport into muscle cells. Interestingly, activity in the proximal part of the insulin signaling cascade in skeletal muscles from cirrhotic animals was increased. This was shown both by increased activity of the IRS1 associated PI3 kinase and by increased expression and phosphorylation of PKB/Akt, key enzymes in the signal to glucose transport. Expression of the insulin sensitive glucose transporter, GLUT4, was normal so together these results indicate that signaling downstream of PKB/Akt and/or the translocation of GLUT4 is impaired in skeletal muscle for cirrhotic animals.

Conclusion: In conclusion, in an animal model of liver cirrhosis whole body insulin resistance is associated with insulin resistance in skeletal muscles. Unlike other common forms of insulin resistance muscles from cirrhotic animals have increased activity in the proximal insulin signaling cascade. This emphasizes that skeletal muscle insulin resistance associated with liver cirrhosis is a unique entity.

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0602

Investigation of the role of Kv1.3 as a potential regulator of peripheral insulin sensitivity

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Background and Aims: Kv1.3, a shaker-related voltage-gated potassium channel, is highly expressed in the olfactory bulb, and is also expressed in a number of other tissues, including fat and skeletal muscle. Kv1.3 knockout mice weigh significantly less than wild-type littermates, have significantly lower fasting and non-fasting blood insulin levels and are protected from diet induced obesity. Channel inhibition has been reported to facilitate the translocation of the insulin sensitive glucose transporter, GLUT4, to the plasma membrane and increase glucose uptake in adipose and skeletal muscle tissues. Furthermore, a polymorphism in the human Kv1.3 gene has been associated with decreased sensitivity to insulin. This channel is therefore an interesting potential target for the development of novel anti-diabetic therapies. The aim of this study was to further investigate the link between channel inhibition and increased insulin sensitivity, using Kv1.3 channel inhibitors in both primary and cultured cells.
Materials and Methods: Work was carried out using the H-2K^b-tsA58 cell line, a potential model of skeletal muscle cells, the HL-1 atrial cardiomyocyte cell line, NIH 3T3 cells (American Type Culture Collection number CRL-1658), 3T3-L1 cells (American Type Culture Collection number CL-173) and isolated primary rat cardiomyocytes and adipocytes. Kv1.3 mRNA expression in cultured and primary cells was measured using real-time RT-PCR. The potencies of three Kv1.3 blockers were tested against Chinese Hamster Ovary cells expressing human Kv1.3 using IonWorksHT, an automated patch clamp instrument. Glucose transport into cells was monitored using [^{3}H] deoxy-D-glucose and GLUT4 translocation was followed by immunocytochemical techniques.
Results: Kv1.3 expression at the mRNA level was detected at similar levels in primary rat cardiomyocytes and adipocytes (0.043±0.012 units and 0.044±0.004 units, respectively). Low expression levels of Kv1.3 mRNA were observed in H-2K^b-tsA58 myoblasts (0.008±0.003 units) and expression increased following differentiation into myotubes (0.078±0.009 units). Expression was also detected in NIH 3T3 cells but not in the HL-1 cardiomyocyte cell line or 3T3-L1 cells. The Kv1.3 channel blockers, Margatoxin, PAP-1 and Psora-4 were found to be active against human Kv1.3 expressed in Chinese Hamster Ovary cells, with IC_{50}s of 0.9±0.9 nmol/l, 22.6±8.5 nmol/l and 36.5±14.8 nmol/l, respectively. Surprisingly, however, we saw minimal effects of these channel inhibitors, both in terms of glucose uptake and GLUT4 translocation, in cells where Kv1.3 mRNA was detected.
Conclusion: Work using transgenic animals has implicated a role for Kv1.3 in regulating peripheral insulin sensitivity. However, despite confirmation of inhibitor activity and channel expression, our present studies, within the limitations of currently available Kv1.3 blockers, have shown only small effects of these inhibitors on cells from insulin sensitive tissues.
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0603

Ras associated with diabetes (RAD) modulates inducible nitric oxide synthase (iNOS) activity in vascular smooth muscle cells (vSMC) from diabetic rats

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Background and Aims: In diabetes, inflammation and increased oxidative stress could induce iNOS expression contributing to vascular damage. Rad, a G protein overexpressed in diabetic animals, binds calmodulin (CaM) in a Ca^{2+}-dependent manner. Since Ca^{2+}-CaM can modulate iNOS activity, aim of this study was to investigate the relationships between iNOS activity and Rad expression in vSMC from DR.
Methods and Results: We measured Rad mRNA levels (Real Time PCR), iNOS expression (RT-PCR, Western Blot) and activity (radiometric technique) and nitrotyrosine levels (immunofluorescence) in cultures of aortic vSMC from 10 diabetic (90% pancreatectomy, DR) and 10 control (sham surgery, CR) rats, after 24 hrs incubation with 20 microg/ml LPS. LPS increased iNOS mRNA and protein levels to the same extent in CR and DR, while iNOS activity was about 7 folds greater in DR. Notwithstanding increased iNOS activity, cGMP levels were not different between DR and CR cells: DR cells, however,

exhibited increased nitrotyrosine levels. As to the effect of LPS on RAD expression, exposure to LPS was followed in CR by a rapid increase in RAD, whereas in DR RAD mRNA levels decreased significantly.

Conclusions: In DR increased CaM availability, possibly due to inhibition of RAD expression, may increase iNOS activity. However, the lack of i.c. cGMP increase and the enhanced nitrotyrosine generation in DR suggest that most of the NO produced in pro-oxidant milieu gets converted into peroxinytrite in these cells. Rad potential role in modulating iNOS activity in diabetes might provide new insight in the mechanisms linking diabetes and atherosclerosis.

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0604

Modulation of hENPP1/PC1 gene expression by heat shock protein 70

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Background and Aims: The ectonucleotide pyrophosphatase phosphodiesterase 1 (ENPP1, also known as plasma cell glycoprotein 1, PC-1) is a class II transmembrane glycoprotein that directly interacts with and inhibits insulin receptor phosphorylation, thus decreasing insulin signaling and action. ENPP1/PC1 is over-expressed in skeletal muscle, adipose tissue and cultured skin fibroblasts of insulin resistant individuals who are not yet obese nor diabetic, thus suggesting that ENPP1/PC1 over-expression is an early, intrinsic defect in human insulin resistance. Previous data indicate that 3′ untraslated region (3′UTR) regulatory elements may modulate gene expression through the regulation of mRNA stability of ENPP1/PC1. The aim of the present study was to investigate whether potential trans-acting cytosolic factors bind to the 3′UTR of ENPP1/PC1, thus modulating its expression

Materials and Methods: RNA probes and REMSA analyses.

The most conserved region of the *in vitro* transcribed ENPP1/PC1 3′UTR, identified by in silico analysis, was used as DNA template for transcribing the RNA probe. Cytoplasmic extract of HEK293 cells and 32P-radiolabeled RNA were incubated in appropriate conditions and the reactions were subjected to 5% nondenaturing PAGE and visualized by autoradiography.

Isolation of ENPP1/PC1 RNA binding protein. High molecular weight complexes were successively excised and eluted and separated on 10% SDS-PAGE: The identified band was eluted and analyzed with tandem mass spectrometry. Analysis of mRNA stability in HEK293 cells. HEK293 cells were treated with antisense Hsp70 oligonucleotide at different time and the occurred silencing was analyzed by western blot. ENPP1/PC1 mRNA half-life (after transcription inhibition by Actinomycin-D) in HEK293 cells treated or not with antisense oligonucleotides was evaluated by RT-PCR on ABI Prism SDS 7000 after total RNA extraction at different time points. HEK293 cells were transfected with pENPP1/PC1 cDNA and its mRNA content was measured after treating or not the cells with Hsp70 antisense oligonucleotide.

Results: REMSA using the most conserved region of the ENPP1/PC1 3′UTR, demonstrated the formation of an abundant high molecular mass RNA-protein complex, thus suggesting the presence of mRNA-binding protein/s in HEK293 cell cytosolic. Tandem mass spectrometry analysis of the high molecular weight complex identified the heat shock protein 70 (Hsp70), as a ENPP1/PC1 3′ UTR binding protein. This result was confirmed by supershift analysis with two different antibody recognizing two different epitopes of HSP70 protein.

The role of Hsp70 in the regulation of ENPP1/PC1 expression was investigated using siRNA-based suppression of Hsp70 and then evaluating ENPP1/PC1 mRNA stability in HEK293 cells after transcription inhibition. Down-regulation of Hsp70 expression by siRNA was paralleled d by a significant decrease of ENPP1/PC1 mRNA stability ($p<0.01$). Moreover, in HEK293 cells transfected with ENPP1/PC1 cDNA, Hsp70 siRNA-based suppression caused a significant reduction (80%; $p=0.001$) in the ENPP1/PC1 mRNA levels as compared to compared to Hsp70 expressing cells.

Conclusion: We identified Hsp70 as ENPP1/PC1 3′UTR binding protein. This specific binding increases ENPP1/PC1 mRNA stability and levels. Our findings suggest a pathogenic role of Hsp70 in insulin resistance through the modulation of ENPP1/PC1 expression.

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0605

Human insulin increases the outgrowth of circulating progenitor cells via IGF-1 receptor dependent MAPKinase activation

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Background and Aims: It has been shown that patients with both diabetes type 1 and 2 have impaired outgrowth and function of endothelial progenitor cells (EPC), which are known to take part in angiogenesis and vascular repair. In patients with poorly controlled type 2 diabetes insulin therapy leads to an increased number of circulating EPC. Therefore, we studied the effect of human insulin and its analogues on outgrowth and cellular signaling cascades in EPC, isolated from healthy volunteers.
Materials and Methods: EPC derived from peripheral blood mononuclear cells (PBMC) of healthy volunteers were grown in medium supplemented with different concentrations of human insulin and insulin analogues in the absence or presence of either IGF-1 receptor- and insulin receptor-inhibitors or specific signal transduction inhibitors. EPC outgrowth was studied using a standardized proliferation assays. Underlying signaling cascades were identified by PCR- based microarrays, RT-PCR and Western Blot assays.
Results: Human insulin significantly increased the formation of EPC colony forming units in a dose-dependent manner with a half maximal effect at 0,25 mU/mL. The only insulin analogue affecting the outgrowth of EPC to a similar extent as insulin was glargine. Incubation of EPC with neutralizing IGF-1 receptor antibodies suppressed EPC proliferation below baseline levels, while inhibition of the insulin receptor had no effect on EPC outgrowth. Insulin dependent EPC proliferation was mediated by IGF-1 receptor dependent downstream signals via ERK1/2, p38 MAPK and JK 2.
Conclusion: Human insulin stimulates the outgrowth of circulating EPC via IGF-1 receptors with downstream involvement of MAPKinase pathways. This might imply a potential role of these signaling cascades as therapeutic targets for modulation of EPC-based vascular regeneration in diabetes.

0606

Binding kinetics of insulin and Insulin-like Growth Factor-II to the insulin receptor isoform A and the dynamics and affinity of interaction between the receptor and Shc after stimulation with these ligands

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Background and Aims: Insulin and the insulin-like growth factors (IGFs)-I and -II bind to and activate receptor tyrosine kinases, thereby triggering a network of signaling pathways. Physiological and genetic evidence suggests that insulin is primarily a metabolic regulator, while the IGFs are primarily growth promoters.
The insulin receptor exists under two isoforms, A (IR-A) and B (IR-B). Recent studies have shown that IGF-II, besides binding to the IGF-I receptor, also binds with high affinity to the A isoform of the insulin receptor. This binding leads primarily to mitogenic (=growth) effects while insulin binding leads primarily to metabolic effects. This is associated with differential recruitment and activation of intracellular substrates. We and others have shown that insulin analogues with slow dissociation kinetics from the insulin receptor have enhanced mitogenicity. These analogues cause a differential activation of the intracellular substrate Shc in comparison to insulin. Shc is the first step in a mitogenic pathway.
We examined, by Bioluminescence Resonance Energy Transfer, in real time and in living human embryonic kidney cells, the dynamics and affinity of interaction between the IR-A and the receptor substrate Shc, after stimulation with insulin, IGF-I and IGF-II.
These data will be correlated with the binding kinetics of insulin and IGF-II on the IR-A, measured by direct 125I-ligand binding studies on whole cells. For that we are using a fibroblast cell line derived from cation-independent mannose-6-phosphate receptor/IGF-II knock-out mice (gift from Dr. von Figura). We have now stably transfected this cell line to overexpress the IR-A.
Materials and Methods: HEK 293 cells were co-transfected with IR-A-Renilla Luciferase and EYFP-Shc cDNA. Two days later and after addition of coelenterazine, BRET measurements were performed in 96-well microplates and at room temperature, using a Fusion microplate analyzer.
Results: The first results show that the three ligands dose-dependently stimulate the interaction between the receptor and Shc. The maximum BRET signal after stimulation with insulin is reached with 100 nM. For IGF-I 2000 nM was necessary and for IGF-II 500 nM. This is in accordance with the data on the relative binding affinities of the ligands for this receptor.
No difference in affinity between the receptor and Shc was detected after stimulation with the above mentioned maximum concentrations of the three ligands. From the kinetic measurements it could be seen that the time to reach maximal BRET signal with these concentrations is approximately the same for the three ligands and the signals continue at this level for at least 30 minutes.
Conclusion: Neither the kinetics nor the affinity of interaction between the receptor and Shc were different after stimulation with 100 nM insulin, 2000 nM IGF-I or 500 nM IGF-II. These results can therefore not explain the different biological effects after stimulating the receptor with insulin or IGF-II.

However, BRET experiments with EC50 concentrations of insulin, IGF-I and IGF-II, are planned. The time the interaction stays intact after removal of the ligands, after stimulation, will also be investigated.
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0607

Function of the novel protein kinase C-dependent serine 357 phosphorylation of insulin receptor substrate-1 on insulin action in skeletal muscle cells
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Background and Aims: The function of insulin receptor substrate-1 (IRS-1), a key molecule of insulin signaling, is modulated by phosphorylation at multiple Ser/Thr residues. The present work was aimed to study the (patho)physiological function and interplay of novel Ser/Thr phosphorylation sites in IRS-1.
Materials and Methods: To identify Ser/Thr phosphorylation sites in IRS-1, *in vitro* phosphorylation assays were performed using GST-IRS-1 protein fragments and protein kinase C isoforms (PKC). The samples were analyzed by SDS 7.5% PAGE and visualized by autoradiography. Identification of specific sites was done by mass-spectrometry. To monitor phosphorylation of Ser-357 in cellular extracts we prepared a polyclonal phospho-site specific antibody. Furthermore, Ala-357 and Ala-318 mutants were made by oligonucleotide mediated site-directed mutagenesis.
Results: Upon incubation of recombinant IRS-1 fragments with various PKC-isoforms we identified Ser-357 of rat IRS-1 (Ser-362 in human IRS-1) as an *in vitro* PKC phosphorylation site. Using the phospho-Ser-357 specific antibody we observed in C2C12 skeletal muscle cells that Ser-357 is strongly phosphorylated by phorbolester (TPA) and to a lesser extent by insulin stimulation. This phosphorylation is mediated, at least partially, by PKC-δ. Moreover, investigating the physiological function of this site on a key molecule in downstream signaling we found after 5 and 10 min of insulin stimulation, i.e. in the early phase of insulin action, that the activation of Akt/PKB was more pronounced in IRS-1 Ala-357 expressing skeletal muscle cells (simulating the unphosphorylated state) compared to cells expressing wild type IRS-1. But, after 60 min of stimulation, i.e. in the continuing insulin action, no difference in the down regulation of Akt/PKB activity between IRS-1 Ala-357 and wild type IRS-1 expressing cells was detectable. Additionally, the phosphorylation of Ser-357 at the early time points of insulin stimulation (5 and 10 min) is more pronounced if Ser-318 is in the unphosphorylated state (represented by the Ala-318 mutation), giving a hint for an interplay of these two PKC phosphorylation sites in IRS-1.
Conclusion: In summary we could show phosphorylation of the novel Ser-357 site of IRS-1 after stimulation with PKC activators TPA and insulin in skeletal muscle cells. Moreover, this site is, at least partially, phosphorylated by PKC-δ. Phosphorylation of Ser-357 could exert negative effects on insulin signal transduction. In addition we have evidence for an interplay of the PKC phosphorylation sites Ser-357 and Ser-318 of IRS-1.
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PS 41

Insulin action

0608

Fetal and neonatal carnitine deficiency influences development and induces insulin resistance in rat pups
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Background and Aims: During normal development, the successful adaptation of neonates to modifications in nutritional state and to various physiological functions requires important changes in glucose and fatty acid metabolism. Carnitine is an important cofactor in fat metabolism and is crucial in the neonatal period, since mitochondrial beta-oxidation is essential for energy production in newborns. Carnitine deficiency was induced in neonates by administration of sodium pivalate to female rats during pregnancy and lactation to evaluate if carnitine deficiency leads to developmental adaptations in various tissues of the offspring, linked to the insulin resistance observed in adult life.
Materials and Methods: Thirty Sprague-Dawley female rats were divided into three groups of ten animals each, treated from day 14 of gestation until weaning (day 21 after birth) with: 1) 20 mM pivalate in drinking water; 2) 20 mM pivalate plus 40 mM carnitine in drinking water 3) vehicle ($NaHCO_3$ 20 mM in drinking water). Carnitine levels were determined in different tissues of fetuses, dams and

neonates by HPLC-MS. Twelve-day-old rats were orally force-fed a solution containing either glucose (25%) or intralipid (10%), collecting blood samples at 0 and 30 min after load. Glucose, insulin, peptide C, triglyceride and NEFA levels were determined in serum; on days 12 and 21, glycogen and triglycerides were also measured in the liver, with specific histopathology on 21st day only. Pancreas morphology was assessed in tissues from 3-, 12- and 21-day-old pups.

Results: A large reduction of carnitine levels in dams, fetuses and in different neonatal tissues was observed in pivalate group (about 60–80% of controls). On day 12 basal insulin levels were significantly increased in carnitine-depleted pups compared to controls (1.09±0.34 vs 0.69±0.20 ng/ml $p \leq 0.01$); the unchanged peptide C levels in pivalate group support the hypothesis of a decrease in liver insulin clearance rather than an increase in insulin secretion. Moreover, an increase in triglyceride levels and a decrease in glycogen content were also observed in the liver of suckling rats. Thirty minutes after glucose load an increase in glucose levels was evidenced in pivalate group (282±5 vs 233±27 mg/dl; $p \leq 0.01$) with no significant increase in insulin levels. After intralipid load, carnitine-depleted pups showed a reduction in insulin secretion with respect to controls, as evidenced by peptide C levels (0.82±0.20 vs 1.37±0.36 ng/ml, $p \leq 0.05$). In pivalate group, an increase in both insulin content and Langerhans islets size was observed only in pancreas from 21- day-old animals, mostly related to increased intracytoplasmic insulin secretory granules and beta cell hypertrophy. Carnitine supplementation restored carnitine levels in pivalate-treated female rats and pup tissues, preventing a majority of above-mentioned alterations.

Conclusion: This work provides evidence of an insulin resistance status during suckling period in carnitine-depleted rats. Carnitine depletion calls for, in the neonate, additional adaptive strategies, both metabolic and organ-specific, which may be at the base of the insulin resistance observed in adult rats, as previously reported.

0609

The effect of HMG-CoA reductase inhibitor on glucose tolerance and insulin sensitivity in type 2 diabetes mellitus animal model

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Background and Aims: We examined the effects of early treatment with pravastatin on the progression of glucose intolerance and insulin sensitivity in the type 2 diabetes mellitus (DM) animal model.

Materials and Methods: Ten week-old Otsuka Long-Evans Tokushima Fatty (OLETF) rats were used as a model of DM and age-matched Long-Evans Tokushima Otsuka (LETO) rats were used as a normal control. The treated OLETF group was divided into 2 groups, receiving low dose (20 mg/kg) pravastatin (OLETF-Low) and high dose (100 mg/kg) pravastatin (OLETF-High) in drinking water from 10 weeks to 40 weeks of age. At 10, 20, and 30 weeks, we examined body weight and the amount of food intake in all experimental animals. At the same time, glucose tolerance test (GTT) was performed and various biochemical analyses was done. We also performed insulin tolerance test (ITT). Lipid profiles, free fatty acids, adiponectin, leptin and insulin were also checked.

Results: The GTT revealed that OLETF groups showed higher glucose levels and AUCg (area under the curve of glucose) than LETO group at 30 weeks. At 20 weeks, 50% and 50% of untreated OLETF rats were impared glucose tolerance (IGT) and DM, but 75% and 8.3% of treated OLETF rats were IGT and DM ($P<0.01$). At 30 weeks, 30% and 70% of untreated OLETF rats were IGT and DM, but 50% and 50% of treated OLETF rats were IGT and DM ($P<0.01$). At 30 weeks, the free fatty acid level was lower in the treated OLETF groups than OLETF-Control group ($P<0.05$). The HDL-cholesterol level was higher in the OLETF-High group than the OLETF-Control group. The OLETF-Low group showed improved insulin sensitivity than the OLETF-Control group in the ITT. Adiponectin, leptin and insulin level between groups was not significantly different.

Conclusion: Early treatment with pravastatin retarded the progression of glucose intolerancein OLETF rats. The favorable effects were associated with the reduction of serum free fatty acid, elevated HDL-cholesterol, and the improvement of insulin sensitivity.

0610

Insulin delivery to muscle is sensitive to tissue perfusion

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Background and Aims: Muscle at rest is only partly perfused and insulin acts *in vivo* to increase capillary recruitment in muscle of rats and human subjects. In normally responsive rats *in vivo*, insulin's microvascular effect occurs at physiological doses, independent of total

blood flow, and prior to changes in Akt phosphorylation or glucose uptake in muscle. To test the possibility that muscle's response to insulin is limited by its delivery, we have partly occluded the rat hindlimb vasculature with 15 μm MS in a constant-flow perfused hindlimb preparation. We then determined insulin-mediated phosphorylation of Akt on Ser 473 and the increase in glucose uptake due to insulin. Assessment was also made of total available nutritive capacity, oxygen and glucose uptake. Oxygen, glucose and insulin have different diffusibility into muscle and therefore we hypothesize that insulin delivery is the most sensitive to limited perfusion of muscle.

Materials and Methods: 15 μm microspheres (MS) were introduced to partially occlude the microvasculature of the surgically isolated pump-perfused rat hindlimb maintained at constant flow. Muscle samples from MS-injected or control perfusions (vehicle only) were assayed for energy charge (EC), phosphocreatine/creatine ratio (PCr/Cr), Akt Ser 473 phosphorylation (P-Akt/total Akt) and muscle 2-deoxyglucose uptake (R'g). Basal oxygen uptake, as well as nutritive capacity (defined as the difference in oxygen uptake during vasoconstriction by maximally vascular recruiting and derecruiting vasoconstrictors), were determined by arterial-venous difference.

Results: MS (0, 9, 15 and 30×10^6 per 15 g hindlimb muscle) into the pump-perfused (0.5 $ml.min^{-1}.g^{-1}$) rat hindlimb led to increased pressure (-0.5 ± 0.8, 15.9 ± 2.1, 28.7 ± 4.6 and 60.3 ± 9.4 mmHg, respectively) with minimal changes in oxygen uptake. Nutritive capacity was decreased from 10.6 ± 1.0 to 3.8 ± 0.9 $\mu mol.h^{-1}.g^{-1}$ ($P<0.05$) with 30×10^6 MS. EC was unchanged, but PCr/Cr was decreased dose dependently to 61% of basal at 30×10^6 MS. Insulin-mediated increases in P-Akt/total Akt decreased from 2.15 ± 0.35 to 1.41 ± 0.23 ($P<0.05$) and R'g from 130 ± 19 to 80 ± 12 $\mu g.min^{-1}.g$ dry wt^{-1} ($P<0.05$) with 15×10^6 MS; basal P-Akt/total Akt and R'g in the absence of insulin were unaffected.

Conclusion: Partial occlusion of the hindlimb muscle by a defined number and size of MS has no effect on basal glucose uptake, marginally impacted on basal oxygen uptake but markedly decreased the total available nutritive capacity. Insulin delivery to muscle was impaired, indicated by decreased Akt phosphorylation, and resulted in reduced insulin-mediated glucose uptake in muscle. Glucose delivery was sufficient in basal conditions but may be limited during insulin stimulation additionally contributing to the reduced insulin-mediated glucose uptake. Thus insulin-mediated glucose uptake by skeletal muscle cells is critically dependent on tissue perfusion that determines insulin delivery.

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0611

DAPIT, a novel protein up-regulated in insulin-sensitive tissues in streptozotocin-induced diabetes

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Background and Aims: We have previously identified a novel gene product DAPIT (Diabetes Associated Protein in Insulin-sensitive Tissues) differentially expressed in insulin-sensitive tissues in streptozotocin-induced diabetes. DAPIT transcript was homologous with wide range of species and ESTs. DAPIT located inside Usmg5 gene in rat chromosome 1 and in unidentified areas of chromosomes 8 and 10. The amino acid sequence of DAPIT was similar to that of a putative protein of *Drosophila melanogaster* and to a cyclic AMP (cAMP)-generating peptide isolated from the flesh fly *Neobellieria bullata*. Our aim was to study DAPIT expression in the insulin-sensitive tissues and in the cell culture systems. We also studied DAPIT effect on cAMP level in DAPIT-transfected cells.

Materials and Methods: By immunohistochemistry and Western blot was studied the DAPIT tissue expression in insulin-sensitive tissues of control and streptozotocin-diabetic rat. By Western blot was studied DAPIT expression in serum free culture medium of rat (L6) and mouse (C2C12) muscle cells, human hepatocytes (HepG2) and kidney cells (HEK293T) stably transfected by DAPIT. By enzyme immunoassay was measured the cellular effect of DAPIT on cellular cAMP level in DAPIT-transfected cells.

Results: The immunohistochemical studies revealed notable DAPIT expression in skeletal and cardiac muscle, adipose tissue, liver and various structures of brain of normal subjects. The protein expression in insulin-sensitive tissues exhibited clear up-regulation of DAPIT in diabetic myocardium, skeletal muscle and adipose tissue but down-regulation in liver. The cell culture studies revealed that native mouse and rat muscle cells, human hepatocytes and DAPIT-transfected cells secrete DAPIT into culture medium. The cellular DAPIT did not affect in cellular cAMP level in over-expression system.

Conclusion: DAPIT shows the expected differential expression in control and in diabetic insulin-sensitive tissues. The cell culture studies and immunohistochemistry let us believe that DAPIT is secreted. The secreted DAPIT effect in secreted cAMP level remains still unknown.

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0612

Antioxidative therapy improving insulin resistance caused by elevated FFAs concentration

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Background and Aims: Elevated circulating FFAs concentration, a characteristic of metabolic syndrome, may induce systemic oxidative stress. Here we report that increased oxidative stress caused by elevated blood FFAs concentration is an important pathogenic mechanism of insulin resistance and antioxidative treatment can improve insulin sensitivity via restoration of normal reactive oxygen (ROS) production.
Materials and Methods: Male S-D rats, 6~8 weeks in age, were assigned to three groups and all underwent 6 hr iv infusions in each group: Controls (C, n=6): saline; FFA (n=6): 20% intralipid1.8 uL/min+heparin 0.72 IU/min; Intervention (I, n=6): GSH (1.2 mg/kg/min) and lipid/heparin. Serum FFAs level and GSH/GSSG ratio both in blood and erythrocyte were measured at baseline and after infusion. The euglycemic hyperinsulinemia clamp study was conducted at the last 2 hours of infusion to evaluate the peripheral insulin sensitivity.
Results: Serum FFAs levels at baseline were similar in the various groups and they were 3 ~5 times higher in FFA group after infusion when compared with C group and they were intermediate between C and FFA group in I group (C: 340.2±103.6 μmol/L, FFA: 1146.4±336.6 μmol/L, I: 620.3±101.4 μmol/L, $P<0.05$). In FFA group the GSH/GSSG ratio both in serum (92.1±10.2 vs. 62.3±7.8 $p<0.05$) and erythrocytes (74.8±8.4 vs. 38.2±4.5 $p<0.01$) were remarkably decreased after infusion. In I group the serum GSH/GSSG ratio also reduced after infusing (89.7±9.3 vs. 75.8±9.3 $p<0.05$), but it only had little change in erythrocytes (71.3±7.3 vs. 72.5±7.1 $p>0.05$). The glucose infusion rates (GIR) during clamp study were 28.6±2.7 mg/kg/min (C group), 21.6±2.7 mg/kg/min (FFA group, vs. C, $p<0.01$) and 25.3±3.1 mg/kg/min (I group, vs. FFA, $p<0.01$) respectively. The values of GIR were positively correlated with GSH/GSSG ratio in erythrocyte ($r=0.92$, $p<0.01$).
Conclusion: The elevated circulating FFAs levels can decrease GSH production through interrupting cells pentose phosphate pathway and lead to insulin resistance. Since there is positive correlation between GIR and GSH/GSSG ratio in erythrocytes and supplement with GSH has beneficial effects on insulin sensitivity and FFAs concentration, the present study suggests that antioxidative treatment may improve insulin resistance through enhancing mitochondria function to deal with elevated FFAs concentration.
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0613

Defects of neuronal NO synthase activity in skeletal muscle of obese insulin resistant Zucker fa/fa rats is due to abnormal expression of the enzyme and its protein inhibitor PIN

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Background and Aims: Skeletal muscle is known to express a variant of neuronal NO synthase (nNOS), nNOSμ, which modulates contractile force and glucose utilization in the myocytes. The importance of such a control is suggested by the muscle and liver insulin resistance occurring in mice lacking nNOS and eNOS genes. Skeletal muscles also express the protein inhibitor of nNOS, PIN, that inhibits nNOS dimerization and catalytic activity. If a defect of the NOS pathway seems to be responsible for insulin resistance in type 2 diabetes, the reasons for remain to be determined. Our aim is thus to identify the nNOS abnormalities present in the skeletal muscle of a rat model of insulin resistance, the obese Zucker fa/fa rat. We therefore studied nNOS expression at the mRNA and proteic levels, its dimerization state as well as PIN expression in the gastrocnemius skeletal muscle of fa/fa rats.
Materials and Methods: nNOS and PIN expression has been analysed by quantitative RT-PCR as well as by Western blotting using specific monoclonal antibodies. Dimerization of nNOS has been studied by low temperature SDS-PAGE, followed by a Western blotting.
Results: We observed a 50% decrease in nNOS expression, in Zucker fa/fa versus control fa/+rats, that affects the whole protein as well as the two splice variants of the enzyme expressed in skeletal muscle. Surprisingly, no changes in nNOS mRNA were observed by quantitative RT-PCR, suggesting that an abnormal nNOS degradation could occur in the skeletal muscle of fa/fa rats. In addition, no changes in the dimeric state of nNOS could be detected. Concerning PIN, we also observed a 75% increase of PIN mRNA, and a 100% increase in the protein level.
Conclusion: The decreased catalytic activity of nNOS observed in skeletal muscle of obese insulin resistant fa/fa rats, is related to a decreased level of nNOS as well as an increased expression of its inhibitor PIN.

0614

Endothelin-1 infusion *in vivo* decreases insulin clearance and causes acute muscle insulin resistance through impaired capillary perfusion

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Background and Aims: Elevated endothelin-1 (ET-1) levels are a common characteristic of many disease states including hypertension, artherosclerosis, peripheral vascular disease, obesity and type 2 diabetes. ET-1 is a potent vasoconstrictor mediating its effects through the ET_A receptor found on smooth muscle cells and may also have metabolic effects through similar receptors on skeletal muscle and adipocytes. The aim of this study was to test the effect of ET-1 infusion (both alone and in conjunction with insulin infusion) *in vivo*, on skeletal muscle blood flow and capillary perfusion, insulin mediated glucose uptake, glucose infusion rate (GIR) and insulin clearance.
Materials and Methods: Anaesthetised Hooded Wistar rats were infused with ET-1 (50 pmol.min^{-1}.kg^{-1}×2.5 hr) alone (ET), or 30 minutes prior to a hyperinsulinaemic euglycemic insulin clamp (10 mU.min^{-1}.kg^{-1}×2 hr, ETINS10). 10 mU.min^{-1}.kg^{-1} (INS10) or 15 mU.min^{-1}.kg^{-1} (INS15) insulin clamps were performed after 30 minutes of saline infusion as reference experiments. Capillary perfusion was measured via 1-methylxanthine metabolism and muscle glucose uptake by 2-deoxy-D-[1-^{14}C] glucose (R'g). Insulin clearance was determined from the rate of clearance of fluorescein isothiocyanate (FTIC) labelled insulin administered as a bolus 45 minutes before the end of a saline or ET-1 experiment.
Results: ET-1 infusion alone and during insulin infusion significantly (P<0.05) increased plasma endothelin concentrations by approximately seven fold to 129±7 and 140±6 pmol.L^{-1} respectively and blood pressure by approximately 20% (P<0.05). ET-1 alone had no effect on femoral blood flow (FBF), capillary perfusion or glucose uptake, but did cause a significant (P=0.02) decrease in insulin clearance from 0.35±0.6 to 0.19±0.02 ml.min^{-1}. ET-1 infusion during insulin clamp resulted in significantly higher plasma insulin levels (INS10: 2120±190 pmol.L^{-1}; ETINS10: 4740±910 pmol.L^{-1}), equivalent to a 15 mU.min^{-1}.kg^{-1} clamp (4920±190 pmol.L^{-1}). C-peptide concentrations remained unchanged throughout the experiments. Both insulin doses significantly (P<0.05) increased FBF, muscle capillary perfusion and glucose uptake. The presence of ET-1 blocked the stimulatory effect of insulin on FBF, and capillary perfusion (P<0.05). GIR decreased (compared to 15 mU.min^{-1}.kg^{-1} infusion) from a steady state infusion of 31.2±1.7 to 22.4±1.2 mg.min^{-1}·kg^{-1} (P<0.05) and muscle glucose uptake (represented by the calf muscle R'g) decreased from 31.5±1.7 to 15.4±2.7 μg.g^{-1}.min^{-1} (P<0.05).
Conclusion: This study shows that ET-1 decreases whole body insulin clearance thereby augmenting plasma insulin levels during insulin clamps. At similar levels of hyperinsulinaemia, ET-1 blocks insulin's haemodynamic effects, particularly capillary perfusion and decreases muscle glucose uptake and whole body glucose clearance. Based on previous studies, it would seem likely that the impairment of capillary perfusion accounts for the acute muscle insulin resistance
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0615

Trans fatty acid feeding leads to insulin resistance and the induction of obesity phenotypes in rats

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Background and Aims: In rodents, high fat diets induce body weight (BW) gain, fat mass accumulation, and insulin resistance (IR) relative to diets lower in fat. The extent to which these phenomena are observed differ with respect to dietary fat type. *Trans* fatty acids (TFA) have a negative impact on blood lipid profiles, but their effects on BW and insulin action are equivocal. Here we test the hypothesis that even a low fat diet enriched with TFA would induce an increase in BW gain and worsen insulin action through effects on tissue lipid profiles.
Materials and Methods: Male 8 wk old Sprague-Dawley rats were fed a 10% saturated fat (LSF), 10% TFA (LFT), or 45% saturated fat (HFS) diet for 8 wk. At 0, 3 and 6 wk, *in vivo* magnetic resonance spectroscopy (MRS) assessed intramyocellular lipid (IMCL) and intrahepatic lipid (IHL) content. During wk 8, euglycemic-hyperinsulinemic clamps determined whole body insulin sensitivity. Ex vivo MRS determined tissue biochemistry following clamp studies.
Results: At wk 6, LFT and HFS groups weighed significantly more than LFS group (510±13, 534±15, 479±16 g; respectively, P<0.05). We used $\Delta_{6\ \text{wk-baseline}}$ total fat mass/body weight ratio as a marker of obesity. Adiposity increased most in HFS, then LFT (LFT 0.07±0.01, HFS 0.08±0.02, LFS 0.04±0.01; P<0.05). When considered by fat depot, visceral fat mass increased significantly more in LFT and HFS (LFT 0.042±0.01; HFS 0.045±0.01; LFS 0.024±0.003; P<0.05), but subcutaneous fat increased significantly more in HFS (LFT

0.03±0.004; HFS 0.05±0.01; LFS 0.02±0.005; $P<0.05$). At 6 wk, % IHL in LFT and HFS was 10.6±1.4, 10.3±1.2, respectively, ~6-fold higher than LFS ($P<0.001$). At 8 wk, whole body insulin action among groups were similar (LFT glucose infusion rate (GIR) 12.5±1.7; HFS GIR, 12.3±2.6, LFS GIR 16.5±2.7 $mg \cdot kg^{-1} \cdot min^{-1}$; $P=0.3$). Basal glucose R_d was similar among diets, while insulin-stimulated glucose R_d was significantly lower ($P<0.05$) in HFS group relative to the other groups and there was a trend ($P<0.06$) for LFT to have lower R_d than LFS group (LFT 12.7±1.6; HFS 11.2±1.7; LFS 17.4±1.8 $mg \cdot kg^{-1} \cdot min^{-1}$). These suggest that both LFT and HFS diets induced peripheral IR. The diet- time interaction for IMCL/tCr was significant in *tibialis anterior* (*TA*) (P<0.001) such that HFS diet had an approximate 2-fold increase in IMCL/tCr ratio at both 3 and 6 wk (3 wk: LFT 1.2±0.4; HFS 2.9±1.3; LFS 1.2±0.7; 6 wk: LFT 1.5±0.8; HFS 2.6±1.1; LFS 1.3±0.5). Diacylglycerol (DAG) concentration in the TA muscle trended ($P=0.06$) to be higher in LTF and HFS relative to the LFS (0.03±0.007, 0.03±0.003, 0.009±0.002% total fatty acid content; respectively).Thus, decreased glucose Rd in the LFT group was independent of an increase in IMCL but was related to increased DAG.

Conclusion: We have shown for the first time that a low fat diet enriched with TFA increases visceral adiposity and intrahepatic lipid accumulation and decreases skeletal muscle insulin sensitivity. Thus a diet low in fat but enriched in TFA provides a model to understand the early physiologic processes implicated in the development of skeletal muscle IR involving increased DAG concentration, prior to the onset of whole body IR. The removal of even small quantities of TFA from the diet would therefore be prudent when considering the etiology of IR and obesity.

0616

Nitric oxide resistance in insulin resistant states: the model of vascular smooth muscle cells from the obese Zucker rats

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Background and Aims: Obesity, both in the absence and in the presence of diabetes, is characterized by a reduced synthesis and action of nitric oxide (NO), playing a role in the pathogenesis of arterial hypertension and atherosclerosis, because NO induces vasodilation and inhibits multiple steps of the atherogenic process. NO mainly acts by inducing the synthesis of guanosine 3′,5′ cyclic monophosphate (cGMP), which exerts a wide spectrum of biological actions by activating the cGMP-dependent protein kinase (PKG).
It has been demonstrated that insulin, via the NO/cGMP pathway, increases Vascular Endothelial Growth Factor (VEGF) synthesis in aortic Vascular Smooth Muscle Cells (VSMC) from lean Zucker fa/+rats by activating both the Phosphatidylinositol-3-Kinase (PI3-K/Akt) and the Mitogen Activated Protein Kinase (MAPK) pathways, and that this NO-mediated insulin effect on VEGF is blunted in VSMC from obese Zucker fa/fa rats, with the possible consequence of a reduced collateral vessel formation. In the present study, we aimed at evaluating whether the NO/cGMP signaling through PI3-K and MAPK pathways is impaired in VSMC from obese Zucker fa/fa rats, a classical animal model of insulin-resistance.
Materials and Methods: In aortic VSMC from lean, insulin-sensitive Zucker fa/+rats and from obese, insulin-resistant Zucker fa/fa rats, obtained in primary culture in our laboratory, we measured the influence of the NO donor DETA NONOate and of the cGMP analog 8-Bromo-cGMP on the activation of the PI3-K/Akt pathway -by evaluating Akt phosphorylation- and of the MAPK pathway -by measuring the phosphorylation of ERK-1 and ERK-2 (western blots, n=6, m±SEM).
Results: In VSMC from Zucker fa/+rats DETA NONOate (500 microM) and 8-Bromo-cGMP (500 microM) increased the phosphorylation of Akt (at 2 hours of incubation, p=0.0001 and p=0.007) and of ERK-1 and ERK-2 (at 2 hours of incubation, p=0.0001 and p=0.013 for ERK-1; p=0.0001 and p=0.019 for ERK-2). A significant activation of PI-3K and MAPK pathways was induced by DETA NONOate and 8-Bromo cGMP also in VSMC from Zucker fa/fa rats (p=0.03–0.007), but the extent of activation was significantly lower than in Zucker fa/+rats. In particular, a) at 2 hours of VSMC incubation with DETA NONOate: i)phosphorylation of Akt was 149.1±12.9% of baseline in lean vs 119.56±4% in obese Zucker rats (p=0.0001); ii)phosphorylation of ERK-1 was 226.0±15.8% of baseline in lean vs 118.9±6.9% in obese Zucker rats (p=0.0001); iii) phosphorylation of ERK-2 was 164.8±5.6% of baseline in lean vs 119.2±6.8% in obese Zucker rats (p=0.0001); b)at 2 hours of VSMC incubation with 8-Bromo-cGMP: i)phosphorylation of Akt was 174.4±8.5% of baseline in lean vs 115.4±8.6% in obese Zucker rats (p=0.0001); ii)phosphorylation of ERK-1 was 169.2±19.8% of baseline in lean vs 118.9±6.4% in obese Zucker rats (p=0.0001); iii)phosphorylation of ERK-2 was 168.2±21.4% of baseline in lean vs 128.5±8.6% in obese Zucker rats (p=0.0001).
Conclusions: The NO and cGMP- induced activation of PI3-K and MAPK pathways is impaired in VSMC from a classical animal model of insulin resistance, the obese

Zucker fa/fa rat, providing new insights into the molecular mechanisms involved in the resistance to NO occurring in the insulin-resistant states, which likely plays a role in their increased cardiovascular risk.
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PS 42

Insulin secretion I

0617

Insulin secretion and insulin action in individuals with different categories of glucose tolerance. The GENFIEV study

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Background and Aims: The GENFIEV (Genetics, PHYsiopathology and Evolution of Type 2 diabetes) is a multicenter study recruiting individuals with IFG and/or IGT to identify phenotypic and genotypic features allowing identification of subjects at high risk for Type 2 diabetes.
Materials and Methods: 1017 subjects have been recruited over 2-yrs. Subjects underwent 75 g OGTT for assessment of plasma glucose (PG) and C-peptide (CP). Fasting insulin was determined as well.
Results: At OGTT, 50% of individuals had NGT, 15% were diabetics, 4% had IFG (110–125 mg/dl), 23% IGT, and 8% IFG+IGT. HOMA-IR increased and the insulinogenic index (DeltaCP30 min/DeltaG30 min) declined as a function of glucose tolerance (ANOVA $p<0.0001$). IFG, IGT, and IFG+IGT subjects all had lower insulinogenic index as compared to NGT and greater than diabetics. To further explore the relationship between glucose tolerance and insulin secretion and sensitivity the population was analyzed based on 2-hrPG as 20 mg/dl glucose increment above an arbitrary value of 100 mg/dl. When the area under the curve (AUC) of CP was considered, a "horse-shoe" shaped relationship was found with a peak AUC for individuals with 160–180 mg/dl 2-hrPG. On the contrary, the AUCCP:AUCG ratio decreased across the 2-hrPG range ($r=-0.37$; $p<0.001$), while HOMA-IR increased ($r=0.28$; $p<0.001$). The ratio between the two parameters, a surrogate of the disposition index, showed a linear decline with increasing 2-hrPG ($r=-0.44$; $p<0.001$). Changes were detected for individuals with NGT, as AUCCP:AUCG ratio ($0.54+/-0.02$ vs. $0.49+/-0.02$, $p<0.075$) and HOMA-IR ($2.01+/-1.2$ vs. $2.8+/-1.7$, $p<0.0001$) were different between individuals with 2-hrPG <100 mg/dl than those with 120–140 mg/dl. Amongst NGT, the ratio between the two parameters dropped by 16% from 2-hrPG <100 to 100–120 mg/dl and by 34% for 2-hr glucose 120–140 mg/dl.
Conclusion: These data, though requiring confirmation in longitudinal studies, suggest that changes in insulin sensitivity and secretion occur in a linear fashion across the different categories of glucose tolerance, and emphasize that major changes in beta-cell performance occur even with modest increases in 2-hrPG within the NGT category.
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0618

The European C-Peptide Trial CPT: comparison of liquid mixed meal and glucagon stimulation assays for the analysis of residual β cell function in type 1 diabetes

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Background and Aims: The European multicentre C-Peptide Trial (CPT) was conducted to compare key characteristics of glucagon and liquid mixed meal stimulated β cell responses in people with type 1 diabetes since residual β-cell function is an important surrogate parameter for β-cell mass in trials aimed at its protection.
Materials and Methods: A total of 118 patients were randomised to three β-cell function tests, each 3–10 days apart. 113 patients 8–40 years of age, 41% female, with diabetes duration 0.75–5 years were included in the analysis. Two baseline blood samples were followed by one sample at 6 min after 1 mg glucagon i.v. (GST,

glucagon stimulation test), or at 10, 20, 30, 60, 90 and 120 min after ingestion of a liquid standard meal (MMTT, mixed meal tolerance test with Boost HP, 6 ml/kg, maximum 360 ml).

Results: The peak C-peptide response to MMTT (geometric mean 0.13 nmol/l, range≤0.01–2.1) was significantly higher than 6 min after glucagon (0.09 nmol/l, range≤0.01–1.37, p<0.0001). The overall intra-individual correlation of 6 min GST with peak MMTT C-peptide concentrations was strong (r=0.90, p<0.0001). MMTT elicited a higher C-peptide response compared to GST in both the tertile with highest basal C-peptide levels as well as in the tertile with lowest basal C-peptide levels (geometric means 0.54 vs 0.38 nmol/l [relative difference 43%] and 0.031 vs. 0.019 nmol/l [relative difference 59%, p=0.46]). A higher C-peptide response to MMTT was also seen in the groups with short as well as with long diabetes duration (9–18 months or 2.5–5 years, geometric means higher by 40% or 27%, p=0.45).

Conclusion: Consistently higher C-peptide responses were observed during MMTT than GST, the close correlation being independent of diabetes duration or basal C-peptide levels. Different kinetics in the loss of β-cell function during the first years after diagnosis of type 1 diabetes between many trials applying either MMTT or GST cannot be accounted for by the method of determining residual β-cell function.

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0619

Several defects of β cell function characterise changes in glucose homeostasis: the GENFIEV Study

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Insulin (I) secretion (Isec) *in vivo* during an OGTT is the sum of 2 components: 1. derivative control (sec1: $pmol \cdot m^{-2}$ BSA per mM of glucose (G)): Isec in response to the rate of increase in G; 2. proportional control (sec2): stimulus (G: mM)- response (Isec: $pmol \cdot min^{-1} \cdot m^{-2}$ BSA) curve. The role of sec1 and sec2 in the early changes in G homeostasis still is under debate.

We aimed at assessing sec1 and sec2 in the early changes in G homeostasis, by exploiting part of the baseline cross-sectional data base of GENFIEV, a multicenter national prospective study of the determinants of the progression from pre-diabetes to type 2 diabetes (DM2). We measured sec1 and sec2 during an OGTT (G and C-peptide curves analyzed by an established mathematical model of G-induced Isec) in 366 (49.5% M) subjects (age: 50.0±0.6 yrs; BMI: 29.0±0.4 kg/m^2), divided according to fasting G (normal: NFG; impaired: IFG; diabetic: DFG) and to 2-hour G (normal: NGT; impaired: IGT; diabetic: DGT) in 9 groups. Diabetic subjects were newly daignosed and treatment naive. The DFG/NGT group (n=2) is not included in this analysis. Comparisons were adjusted by age, gender, BMI and fasting insulin, as a proxy of I-resistance. Sec1 was similar in NFG/NGT (1390±98, n=87), IFG/NGT (1267±147, n=39) and NFG/IGT (1595±121, n=59), declined in IFG/IGT (1038±98, n=86, p<0.01), and fell further in NFG/DGT (n=10), IFG/DGT (n=51), DFG/IGT (n=8) and DFG/DGT (n=24) (value ranging between 697±185 and 808±286, p<0.01). In comparison to NFG/NGT (Isec: 105±4.5, 197±9, 545±21, 982±42 at G levels of 4.0, 5.5, 8.0, and 11 mM, respectively), sec2 declined (~22%, p<0.03) in IFG/NGT and NFG/IGT, was further decreased (~41%, p<0.01) in IFG/IGT and NFG/DGT, and fell even more (between 56% and 65%, p<0.01) in IFG/DGT, DFG/IGT and DFG/DGT.

Conclusions: Across the spectrum from normal G homeostasis to newly diagnosed DM2, sec1 shows at least 3 and sec2 shows at least 4 distinct levels of β-cell function, suggesting that the evolution to DM2 crosses several stages of $^{1}_{2}$-cell function, the last of which implies invariably a joint decline in both sec1 and sec2.

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0620

Characterisation of insulin secretory defect in Bangladeshi type 2 diabetic subjects

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Background and Aims: It still remains controversial whether insulin deficiency or insulin resistance is the earliest abnormalities in the natural history of the disease. Considerable heterogeneity seems to exist regarding these abnormalities among various populations. Previous studies suggest that insulin secretory dysfunction is the relatively more prominent defect in Bangladeshi nonobese type 2 diabetic population. The characterization of the defect may provide valuable tools for further understanding of the role of insulin secretory defect in the pathogenesis of type 2 diabetes. The aim of the present study was to investigate the nature of insulin secretory defect in type 2 diabetic subjects in various phases of secretion.

Materials and Methods: Forty-four type 2 diabetic subjects, along with 30 Age-and BMI-matched control subjects, were studied. The diabetic subjects were never treated with insulin. Insulin was measured by chemiluminiscent ELISA. Insulin secretory capacity (%HOMA B) and insulin sensitivity (%HOMA S) were calculated by homeostasis model assessment (HOMA) using HOMA software. First and second phases of insulin secretion were calculated by the following equations: 1st $phase_{est}$=1283+ $1.829.Ins_{30}$-$138.7.Glu_{30}$+$3.772.Ins_0$; 2nd $phase_{est}$=287+ $0.4164.Ins_{30}$-$26.07.Glu_{30}$+$0.9226.Ins_0$

Results: The mean age (years, M±SD) of the patients was 44±7 and that of controls 41±7. BMI (kg/m^2, M±SD) was 25.46±3.26 in patients vs 25.31±3.61 in controls. Fasting serum insulin [Median (range); 53.96 (6.67–162.58) vs 74.55 (6.25–216.89), p=0.0001]; and 30 min after glucose insulin [Median (range); 132.44 (17.78–422.19) vs 544.59 (33.27–1168.64), p=0.013] levels were significantly lower in diabetic as compared to control subjects. The B-cell function was significantly lower in diabetic subjects as compared to the controls [%HOMA B, Median (range); 53.90 (10.40–162.90) vs 163.60 (48.40–381.70), p=0.0001], but no significant difference was observed in case of insulin sensitivity [%HOMA S, Median (range); 79.10 (26.40–226.90) vs 65.20 (21.90–259.90), p=0.088]. The 1st and 2nd phases of insulin secretion were also significantly lower in diabetic subjects compared to control [Phase I: Median (range); 208.49 (−1412–1230) vs 1655.56 (−109–2831), p=0.0001, Phase II: Median (range); 101.23 (−217–341) vs 418.56 (51–679), p=0.0001]. A significant positive correlation was found between BMI and Phase I (r=0.315; p=0.037) and Phase II (r=0.320; p=0.034) of insulin secretion in case of diabetic subjects. No such correlation was observed in case of the control subjects. A significant negative correlation was also observed between insulin sensitivity and BMI (r=− 0.439; p=0.003) in the diabetic subjects.

Conclusion: Nonobese type 2 diabetic patients in Bangladesh have insulin secretory dysfunction and they do not seem to have insulin resistance as a primary pathophysiologic characteristics. The B-cell secretory dysfunction in type 2 diabetics involves both fasting and poststimulatory states; poststimulatory defect, however, is much prominent in comparison to the fasting state. Both 1st and 2nd phases of insulin secretion are affected in type 2 diabetes, but the deficiency in 1st phase secretion seems to be predominant in these subjects.

0621

Pitfalls of the disposition index as an index of beta cell function

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Background and Aims: It is generally agreed that, in healthy subjects some parameters of insulin secretion are reciprocally related to insulin sensitivity. This notion has led to a general paradigm whereby beta-cell function is correctly assessed by calculating the "disposition index" (DI), i.e., the product of insulin secretion and insulin sensitivity. However, the validity of this approach is critically dependent on the assumption that the relationship between insulin secretion and insulin sensitivity is hyperbolic. To test whether this assumption is correct, we used the database of the RISC study, which includes measures of insulin sensitivity and secretion.

Materials and Methods: Insulin sensitivity was measured by the euglycemic clamp and insulin secretion was assessed by two typical parameters, i.e., fasting plasma insulin concentration and the insulinogenic index (i.e, the ratio of incremental insulin to incremental glucose concentration at 30 min into an OGTT). All subjects (506 men/633 women, age 44±8 years, BMI 25±4 $kg{\cdot}m^{-2}$, mean±SD) had normal glucose tolerance on the OGTT. The validity of the hyperbolic assumption was tested by verifying the constancy of DI across the insulin sensitivity span. Subjects were stratified into deciles of insulin sensitivity and the constancy of DI in the groups tested by ANOVA.

Results: DI increased with insulin sensitivity across the entire sensitivity range (p<0.0001), both when DI was calculated using the fasting insulin concentration (~2-fold increase from the lowest to the highest insulin sensitivity decile) and when using the insulinogenic index (~3-fold increase). This artifact derives from the fact that the relationships between insulin sensitivity and secretion are not hyperbolic, but rather power functions with an

exponent>−1. Furthermore, when fasting plasma glucose concentration was stratified into deciles, the DI using fasting insulin was positively related to fasting glycemia ($p<0.00001$), whereas the DI using the insulinogenic index was negatively related to fasting glucose ($p<0.00005$). Thus, DI neither correctly estimates beta-cell function across the insulin resistance range nor is unequivocally predictive of fasting plasma glucose concentrations.

Conclusion: This study shows that the hyperbola paradigm and the DI are not generally valid, and their use may produce significant artifacts if validity is not tested.

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0622

Pancreatic islet blood flow is selectively enhanced by captopril, irbesartan and pravastatin, and suppressed by palmitate

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Background and Aims: The systemic renin-angiotensin system (RAS) plays a crucial role in the regulation of arterial blood pressure. In the pancreas of several species, mRNA encoding angiotensinogen and renin, as well as substantial levels of angiotensin II, have been detected. Ang II has been shown to adversely influence pancreatic and islet blood flow through vasocontractive effects. Also, high affinity binding sites for Ang II were recently localized specifically to islet β-cells by double immunostaining and Ang II was found to block glucose-stimulated insulin secretion, an event fully reversible by losartan. It is thus conceivable that pancreatic Ang II, locally produced by intrinsic RAS, may adversely influence insulin secretion *in vivo*, either directly by suppressing β-cell insulin exocytosis or indirectly through inhibitory effects on islet blood perfusion. This may be of particular importance in diabetic patients since hypertension is markedly overrepresented in these individuals, and angiotensinogen expression seems to be upregulated by hypertension. Hence, many diabetic patients are treated with ACE inhibitors or angiotensin receptor antagonists against their hypertension or as part of a renal protection strategy. An additional hallmark of diabetic cardiovascular risk is hyperlipidemia and elevated serum levels of free fatty acids; consequently many diabetic patients use lipid lowering drugs, most notably statins. Interestingly, both ACE inhibitors or angiotensin receptor antagonists and certain statins have been reported to decrease the risk of developing diabetes in large clinical trials. However, the mechanisms behind these antidiabetic effects remain elusive. We now studied whether these drugs and the fatty acid palmitate influence insulin secretion *in vivo* in rats through effects on islet blood perfusion.

Materials and Methods: Male Wistar rats weighting 300–350 g, with free access to tap water and pelleted food, were used in all experiments. The microsphere technique in combination with a freeze-thawing technique was performed for measurements of pancreatic blood flow and islet blood flow.

Results: Whole pancreatic blood flow was markedly increased by captopril and irbesartan and decreased by palmitate. Islet blood flow was significantly and preferentially enhanced by captopril, irbesartan and pravastatin, and suppressed by palmitate. Both captopril and irbesartan raised serum insulin concentrations significantly. However, glycemia was not affected in any group.

Conclusion: The present study suggests that a local pancreatic RAS and pravastatin may be selectively controlling pancreatic islet blood flow and thereby influencing insulin secretion. The antidiabetic actions of statins and RAS inhibitors noted in clinical trials might in part occur through the beneficial direct islet effects shown here. Conversely, free fatty acids that are elevated in type 2 diabetic patients may contribute to an impaired nutritive islet blood flow and thereby further aggravate the diabetic state by limiting the supply of insulin needed to curb hyperglycemia.

0623

Relationships between insulin secretion after acute intravenous and oral glucose administration from normal glucose tolerance to diabetes

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Background and Aims: It has long been known that first-phase insulin secretion in response to acute intravenous glucose injection (IVGTT) can be abolished in diabetic subjects at a time when their response to oral glucose is maintained. However, the mechanisms underlying this phenomenon are poorly understood.

Materials and Methods: We measured the acute insulin response (AIR) to intravenous glucose and the secretory response to an oral glucose tolerance test (OGTT) in 221 subjects (194 women/27 men; age 37±11 years, BMI 28±5 kg/m^2; mean±SD) spanning the range from normal glucose tolerance (NGT, n=147) to impaired fasting glycaemia/impaired glucose tolerance (IFG/IGT, n=28) to

overt diabetes (T2D, n=46). Beta-cell function parameters from the OGTT, and, in particular, beta-cell glucose sensitivity, were obtained by modelling analysis.

Results: Both AIR and beta-cell glucose sensitivity declined as glucose tolerance worsened ($p<0.0001$ by ANOVA). However, AIR reached a value near zero at a fasting glycaemia of ~7 mmol/L, while beta-cell glucose sensitivity fell continuously throughout the fasting plasma glucose range. Therefore, the relationship between AIR and beta-cell glucose sensitivity, which was strongly linear in NGT ($r=0.58$, $p<0.0001$), became flat at ~7 mmol/L glucose. In addition, for fasting glucose levels above 7 mmol/L, AIR was no longer related to fasting glucose, while a strong relationship between fasting glucose and glucose sensitivity was preserved ($r=0.71$, $p<0.0001$). Similar results were obtained when using 2-hour plasma glucose levels instead of fasting glucose, the glucose threshold being ~13 mM.

Conclusion: AIR vanishes at thresholds of fasting or post-glucose hyperglycaemia before beta-cell glucose sensitivity is severely compromised. Consequently, AIR misjudges beta-cell function in hyperglycaemic states.

0624

Beta cell function and insulin sensitivity in subjects with non-alcoholic fatty liver disease and normal glucose regulation

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Background and Aims: Ever-increasing evidences has suggested that non-alcoholic fatty liver disease(NAFLD) is associated with type 2 diabetes mellitus and insulin resistance(IR). The relationship of NAFLD and beta cell function, however, was seldom presented. This study was to investigate the impact of liver fat content on beta cell function and insulin sensitivity in subjects with NAFLD and normal glucose regulation (NGR).

Materials and Methods: Twenty seven subjects aged 20 to 60 years with NGR (assessed by OGTT) were recruited. They had no history of excessive drinking (less than 40 g per day in males and 20 g per day in females) and were negative in serological analysis of HBsAg and HBC-Ab. Anthropological parameters(BMI, waist circumference, and waist-to-hip ratio) of all participants were collected and liver fat content was evaluated by semi-quantitive CT scan technique. According to liver fat content, subjects with NAFLD were divided into three groups. Liver fat content in subjects of group A(n=6)was no more than 5.0%, group B (n=7)5.1% ~9.9% and group C(n=6)10.0% ~20.0%. Eight healthy subjects were enrolled as normal control group. Beta cell function and insulin sensitivity were assessed by hyperglycemic clamp technique. Area under first phase insulin release curve (AUC_1) and area under second phase insulin release curve(AUC_2) were calculated to analyze beta cell function. Insulin sensitivity was demonstrated by insulin sensitivity index (ISI). All participants signed written informed consents.

Results: There were no significant differences in age, sex, waist circumference, and waist hip ratio among Group A, B, and C. BMI of Group C(30.80±3.43 kg/m^2) were higher than that of Group A (25.86±0.92 kg/m^2) and B(26.42±2.28 kg/m^2) ($p<0.05$) while no significant difference was observed between group A and group B. The AUC_1 was 2.60±0.12 min.mU/L in group A, it increased to 2.81±0.18 min.mU/L in group B($p>0.05$), and returned to 2.70±0.23 min.mU/L($p>0.05$)in group C. The AUC_2 was 4.03±0.28 min.mU/L in group A, increased to 4.20±0.17 min.mU/L in group B, and further increased to 4.41±0.17 min.mU/L in group C(ANVOA: Group A vs Group B, $p>0.05$; Group C vs Group A, $p<0.05$; Group C vs Group B, $p>0.05$).ISI was 25.14±14.77 mg.L/(kg.min.mU) in group A, it decreased to 8.31±2.24 mg.L/(kg.min.mU) in group B, and further descended to 4.57±2.49 mg.L/(kg.min.mU) in group C (ANVOA: Group A vs Group B, $p<0.05$; Group C vs Group A, $p<0.05$; Group C vs Group B, $p>0.05$).

Conclusion: The results suggest that there is a positive correlation between the extent of IR and the level of liver fat content in patients with NAFLD and NGR when their liver fat contents are no more than 20%. A positive correlation may also occur in first phase insulin release of beta cells and liver fat contents in patients with NAFLD and NGR when their liver fat contents are no more than 10%. Beta cell dysfunction, however, may appear in such patients when their liver fat contents increase from 10% to 20%. Controlling liver fat content may benefit improvement of IR and beta cell dysfunction in patients with NAFLD.

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0625

Beta cell dysfunction in IGT and early type 2 diabetes: comparison of surrogate markers with first phase insulin secretion from an intravenous glucose tolerance test

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Background and Aims: Methods to assess β-cell function in clinical studies are limited. The aim of the current study was to compare a direct measure of insulin secretion with indirect markers in relation to glucose tolerance status.
Materials and Methods: In n=1380 individuals from the IRAS (Insulin Resistance Atherosclerosis Study), β -cell function was assessed using a frequently sampled intravenous glucose tolerance test (first phase insulin secretion; AIR), HOMA-B (using the formula, 1985; HOMA-B or the computer program, 1998; HOMA-%B), proinsulin (PI) levels and the PI/insulin ratio (P/I-R). β-cell function was cross-sectionally analysed by glucose tolerance categories [NGT; n=712, IGT; n=353; newly diagnosed diabetes by 2 h glucose criteria from an OGTT; n=80 (DM2 h) or by fasting glucose criteria; n=135 (DMf), or established diabetes on diet/exercise only; n=100 (DM)].
Results: In Spearman correlation analyses (all p-values <0.0001), proinsulin levels were moderately related to HOMA-B and HOMA-%B (r=0.17 and 0.13), and inversely related to AIR (r=−0.21). The PI/I-R was inversely related to HOMA-B, HOMA-%B and AIR (−0.49, −0.51, and −0.27, resp.). AIR was related to HOMA-B and HOMA-% B (r=0.43 and r=0.47). The table shows β-cell function (normalized to NGT) by glucose tolerance category.

	NGT	IGT	DM2 h	DMf	DM
Fasting glucose (mg/dl)	95.3	103.8	110.4	157.8	168.0
Proinsulin	100	148	220	331	323
1/PI	100	67	45	30	31
P/I-Ratio	100	112	133	173	179
1/PIR	100	89	75	58	56
HOMA-B; 1985	100	106	111	65	58
*HOMA-B; 1985	100	81	71	30	27
HOMA-%B; 1998	100	104	106	63	56
*HOMA-%B; 1998	100	87	78	41	38
AIR	100	79	65	38	43
*AIR	100	60	40	23	25

* adjusted for insulin sensitivity, as assessed by HOMA or MINMOD, respectively

Conclusions: Indirect markers of β -cell function were modestly related to each other and to directly measured (first phase) insulin secretion. Markers based on proinsulin levels better discriminated between glucose tolerance categories than HOMA-B, particularly when unadjusted for insulin resistance. All markers markedly underestimated the magnitude of the β-cell defect across declining glucose tolerance status compared to a direct measure of insulin secretion (AIR). Subjects with IGT and early stage type 2 diabetic patients may have markedly more pronounced β-cell defects than estimated from epidemiological studies using indirect markers of β-cell function.
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0626

Effects of exercise on the function and morphology of pancreatic islet cell in insulin resistant rats

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Background and Aims: Insulin resistance is a common pathogenetic feature of type 2 diabetes mellitus. Nondiabetic subjects have the ability to secrete enough insulin as a compensate for insulin resistance,whereas in subjects with type 2 diabetes mellitus, the adaptation is insufficient. Islet dysfunction is a key factor promoting the progression from insulin resistant state to overt type 2 diabetes mellitus. Previous studies have provided evidence that swimming is an effective intervention to improve body's insulin sensitivity. The aim of the study is to observe the effects of swimming on the function and morphology of pancreatic islet cell in insulin resistant rats, and to investigate the relationship between the improvement of insulin sensitivity and the protection of pancreatic islet function.
Materials and Methods: Normal male Wistar rats were randomly allocated into 3 groups: a normal-chow (15.36 kcal/g, 17%fat) group (NC, n=15), a high-caloric and high-fat diet (22.39 kcal/g, 55%fat) group (HF, n=15), and a swimming (50~60 mins/d, 5 days/w) group with high-caloric and high-fat diet(SW, n=15). Insulin resistance was assessed by euglycemic hyperinsulinemic clamp technique. The insulin secretory function of islet was evaluated by intravenous insulin releasing test (IVIRT). Morphological and quantitative analysis was performed on pancreatic tissues stained by double-label insulin and glucagon through immunohistochemistry approach. Proinsulin mRNA was detected by RT-PCR.
Results: Rats taking HF had severe visceral obesity and developed remarkable insulin resistance compared with NC rats (GIR60~120: 7.60±1.29 vs 5.83±0.79, P<0.05), whereas the insulin sensitivity in SW rats had significantly improved (7.40±1.60, P<0.05). Immunohistochemistry showed the insulin relative concentration of β cells in HF decreased,as compare with NC rats (P<0.01). And islet size had an 1.42-fold increased in HF rats (P<0.01). The islet relative β-cell volume was decreased by 33.68% in HF

(P<0.01), whereas the relative α cell volume increased by 57.96%(p<0.01). So there was a 60.47% decrease of the ratio of β to α cells in HF. As compared with HF rats, SW rats had a higher insulin relative concentration of β cells (P<0.01)and lower islet size (P<0.05). Swimming intervention resulted in 34.87% increase in the islet relative βcell volume, and have a 86.32% increase in the ratio of β to α cells. The peak of insulin secretion in IVIRT in NC and SW was on 5 min, whereas that in HF rats was on 10 min. AUC (area under curve) 10~60 of insulin in HF was higher than NC and SW rats (P<0.05). Proinsulin mRNA level had no significant difference among 3 groups.

Conclusion: Long-term high-caloric and high-fat diet results in early impairment of islet morphology and function, and significant insulin resistance as well. It shows in the early course of Type 2 diabetes mellitus the adaptation of islet has already been impaired. By meliorating insulin resistance, exercise can reverse the early change of morphology and function of pancreatic islet, maintain and increase the islet compensation to insulin resistance.

PS 43

Insulin secretion II

0627

Characterisation of a non-genetic non-obese model of type 2 diabetes in mice given streptozotocin and nicotinamide and its application for testing the anti-diabetic properties of a new antioxidant compound

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Background and Aims: On the basis of a diabetic syndrome with reduced beta-cell mass previously obtained in rats, we intended to establish an original model of type 2 diabetes in mice, i.e. in a species of small size, suitable for long-term experimentation of scarcely available or expensive new potential antidiabetic agents. Furthermore, as oxidative stress is considered a major mechanism of progressive beta cell damage in diabetes, we tested in this model the protective effects of a new antioxidant drug.

Materials and Methods: Male and female C57Bl/6 mice were given diabetogenic doses of streptozotocin (STZ) combined with different partially protective doses of nicotinamide (NA) and monitored for glycaemia, insulinaemia, glucose tolerance and pancreatic insulin content. These parameters as well as plasma nitrotyrosine as an index of oxidative stress were also evaluated in a cohort of male STZ-NA mice during a 5-week treatment with a novel antioxidant compound (IACVITA, Medestea).

Results: The combined administration of appropriate doses of STZ and NA to adult mice, in the absence of genetic alterations, resulted in a diabetic syndrome characterized by moderate hyperglycaemia (~ 200 mg/dl in non-fasting animals, which remained stable for approximately two months, progressively increasing thereafter), hypoinsulinaemia, glucose intolerance, growth impairment and markedly reduced pancreatic insulin content (20–30% as compared to controls). Upon treatment with the antioxidant drug, STZ-NA diabetic mice showed significant reduction but not normalization of plasma glucose levels, a slight increase in circulating insulin and a better preservation of residual pancreatic insulin content (controls: 126±5.4 ng/mg pancreas; STZ-NA: 28±4.6 ng/mg; STZ-NA+antioxidant: 56±7.1 ng/mg). Plasma nitrotyrosine levels, markedly enhanced in untreated diabetic mice, were significantly reduced although not fully reversed by the treatment (controls: 0.29±0.03 nM; STZ-NA: 0.79±0.04 nM; STZ-NA+antioxidant: 0.55±0.06 nM). Highly significant correlations were found between plasma nitrotyrosine values and either plasma glucose (r=0.59, p<0.01) or pancreatic insulin content (r=0.70, p<0.01) of all experimental groups.

Conclusion: We have established a novel non-obese non-genetic murine model of type 2 diabetes with reduced pancreatic insulin stores, particularly suitable for pharmacological research in diabetes. In this model, the new antioxidant compound IACVITA was able to partially reverse diabetic metabolic alterations, likely by counteracting beta cell dysfunction and loss associated with oxidative stress.

0628

Regulation of pancreatic beta cell function and viability by adiponectin

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Background and Aims: Obesity is a risk factor for the metabolic syndrome and type 2 diabetes. Adiponectin, a 30 kDa adipocyte secreted peptide has been shown to be anti-atherogenic, and anti-inflammatory, as well as affecting insulin resistance and pancreatic beta-cell function. This study investigated the effects of full length and globular

adiponectin on pancreatic beta-cell viability, and expression of UCP-2, PDX-1 and LPL genes, as well as studying levels of expression of the adiponectin receptor subtypes 1 and 2 (AdR-1 and AdR-2) and their regulation in the pancreatic BRIN-BD11 beta-cell line.

Materials and Methods: Cells were cultured in RPMI-1640 and treated with full length (fAd) or globular (gAd) adiponectin (0–100 nM)+/−50 ng/ml leptin to test effects on cell viability, where an MTS assay was used. For gene expression experiments, 100 nM adiponectin concentrations were used. For AdR expression experiments, cells were treated with rosiglitazone (10 micromolar), leptin (10 ng/ml and 50 ng/ml), oleic acid (50 micromolar), palmitic acid (50 micromolar), elaidic acid (50 micromolar) and the PPAR alpha agonist WY14643 (10 micromolar) for 24 hours. Total cellular RNA/protein was extracted and expression assessed by real-time PCR/Western blotting respectively.

Results: Both fAD and gAD caused significant increases in cell viability, however fAd only was reversed by addition of 50 ng/ml leptin. Expression of AdR- 1 mRNA was 70-fold higher than that of AdR-2 ($p<0.001$) however this was reversed at protein level. Rosiglitazione treatment resulted in a 32% decrease in AdR-2 mRNA expression ($p=0.029$) but had no effect on AdR-1, with palmitic acid having similar effects. Oleic acid treatment conversely resulted in a decrease in expression of both receptor subtypes ($p<0.05$) with WY14643 having similar effects. Leptin had no effect on expression of either receptor type. Neither fAd or gAd affected UCP-2 mRNA expression, however gAd increased LPL expression and PDX-1 expression.

Conclusion: These observations confirm a differential expression of AdR-1 and −2 in pancreatic beta-cells, and suggest a role for adiponectin in beta-cell function and viability which may be altered in obesity and insulin resistant state.

0629

L-[U-^{14}C]glutamine and [U-^{14}C]palmitate metabolism in islets from rats depleted in long-chain polyunsaturated ω3 fatty acids

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Background and Aims: Rats depleted in long-chain polyunsaturated ω3 fatty acids (ω3 rats) present several features of the metabolic syndrome, including obesity, ectopic fat accumulation and insulin resistance. This coincides with an alteration of D-glucose metabolism in pancreatic islets characterized by an impaired response to increases in hexose concentration and low oxidation/utilization ratio. The specificity and possible mechanism of such an alteration were now investigated by comparing the metabolism of L-[U-^{14}C]glutamine and[U-^{14}C]palmitate in islets from normal and ω3 rats.

Materials and Methods: The production of $^{14}CO_2$ by islets from female normal and ω3 rats was measured upon either exposure to exogenous L-[U-^{14}C]glutamine and [U-^{14}C]palmitate or after prelabelling of the islets with these nutrients.

Results: Despite comparable uptake of the amino acid, the oxidation of exogenous L-[U-^{14}C]glutamine (pmol/islet per 30 min) was higher ($p<0.001$) in ω3 rats (21.5±0.8; n=83) than in normal rats (16.7±0.7; n=30). The output of $^{14}CO_2$ from prelabelled islets, as measured in the presence of either D-glucose (8.3 mM) or metabolic poisons, when expressed relative to the corresponding value found in the absence of these agents, was comparable in ω3 and normal rats, with mean respective values of 110.8±3.9% (n=74; D-glucose) and 24.6±1.7% (n=72; metabolic poisons). Over 90 min incubation, both the oxidation and uptake of exogenous [U-^{14}C]palmitate were significantly higher in ω3 than normal rats. Even the ratio between these two variables remained higher ($p<0.05$) in the ω3 rats (3.14±0.30%; n=39) than in normal rats (2.26±0.26%; n=26). Moreover, relative to the value found in the absence of exogenous nutrients, the output of $^{14}CO_2$ from prelabelled islets incubated in the presence of D-glucose (8.3 mM) was higher ($p<0.001$) in ω3 rats (117.9±6.2%; n=29) than in normal rats (78.5±5.5%; n=18).

Conclusion: These findings document that the impaired catabolism of D-glucose in islets from ω3 rats represents a specific feature, contrasting with increased oxidation of L-[U-^{14}C]glutamine and [U-^{14}C]palmitate. The increased uptake and oxidation of [U-^{14}C]palmitate by the islets of ω3 rats is consistent with both an accelerated clearance of circulating fatty acids in these animals and the proposal that the impairment of D-glucose metabolism in the islets of ω3 rats may be attributable to altered glucokinase kinetics as a result of a high acyl-CoA content of islet cells.

0630

Effect of increased L- alanine, L- glutamine and L-aspartate concentrations on INS- 1E cells over- expressing Aralar1

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Background and Aims: NADH shuttles transport reducing equivalents from the cytosol to the mitochondria. In pancreatic beta cells, the NADH shuttle system plays an essential role in coupling glucose metabolism to insulin secretion by delivering glycolysis- derived electrons to the electron transport chain in the mitochondria, which then, through ATP production and subsequent calcium rise, initiates insulin exocytosis. In beta cells, the NADH shuttle system generally consist of the glycerophosphate and the malate- aspartate shuttle. In beta cells, the latter functionally predominates and plays a crucial role in both mitochondrial metabolism and cytosolic redox state. One key constituent of the malate- aspartate shuttle is Aralar1, a Ca^{2+} sensitive isoform of the aspartate/ glutamate carrier 1 (AGC1) known to have a potentiating effect on glucose- stimulated insulin secretion. We over-expressed Aralar1 by means of a recombinant adenovirus in the INS- 1E beta cell line and investigated cell metabolism and nutrient- stimulated insulin secretion, in particular the impact of L- alanine, L- aspartate and L-glutamine.

Materials and Methods: INS- 1E cells were cultured for 72 hours prior to transduction with AdCA- Aralar1 and AdCA- LacZ and then incubated with L- alanine (2 mM), L- aspartate (2 mM) and L- glutamine (10 mM) respectively. Cell viability was determined using a WST-1 assay. Insulin secretion was measured by radioimmunoassay. Mitochondrial membrane potential was determined upon stimulation with 15 mM glucose using 123- rhodamine as a probe. NADH levels were measured as autofluorescence.

Results: Insulin secretion measured in Aralar1 over-expressing cells cultured with L- alanine was enhanced compared to control cells cultured without L- alanine, resulting in a 20% increase upon 15 mM glucose stimulation and a 25% increase at 2.5 mM glucose (for both conditions $p<0.05$). Both L- alanine and control cells gave a 5.5- fold increase in insulin secretion relative to basal 2.5 mM glucose levels. Insulin secretion in Aralar1 over- expressing cells was decreasedby 2 mM L- aspartate to almost 50% of the control cells ($p<0.05$). Insulin secretion in transduced cells remained unaltered by L-glutamine. Cell metabolism, as assessed by mitochondrial membrane potential and NADH levels, was also potentiated by L- alanine ($p<0.05$), inhibited by L- aspartate.

Conclusion: Cell metabolism and insulin secretion in INS-1E cells over- expressing Aralar1 was enhanced by 2 mM L- alanine but decreased by L- aspartate. Our results indicate beneficial effects of L- alanine for metabolic secretion coupling.

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0631

The ability of non-glucose nutrients to modulate postprandial insulin and glucagon secretion is intact in people with pre-diabetes

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Background/Aim: Although there is extensive data indicating that insulin secretion is impaired in subjects with pre-diabetes, it is not known if the ability of non-glucose nutrients (e.g. protein and fat) to modulate glucose induced insulin and glucagon secretion also is impaired.

Methods: 32 subjects (16F/16M; 54±1 years; BMI: 31.3±0.9 kg/m^2) with impaired fasting glucose (IFG: 5.5–6.9 mmol/l) and 17 subjects (11F/6M; 50±2 years; 27.7±0.8 kg/m^2) with normal fasting glucose (NFG: <5 mmol/l) ingested in random order on separate occasions 75 grams of glucose (OGTT) or a mixed meal (45% CHO, 15% protein and 40% fat) also containing 75 grams of glucose. Plasma glucose, insulin, C-peptide and glucagon concentrations were measured at frequent intervals for 240 minutes.

Results: Fasting plasma glucose concentrations did not differ on the OGTT and meal study days in either the IFG (5.7±0.1 vs. 5.8±0.1 mmol/l) or NFG (4.9±0.1 vs. 4.9±0.1 mmol/l) groups. In addition, although 2 hour glucose concentrations were lower after meal than OGTT in the NFG (6.1±0.3 vs. 6.9±0.1 mmol/l; p=0.01) and IFG (8.9±0.5 vs. 9.7±0.4 mmol/l; p=0.01) groups, the glucose area above basal did not differ in either the IFG (522±51 vs. 562±54 mmol/4 hrs) or NFG (353±23 vs. 348±45 mmol/4 hrs) groups. Despite comparable glucose areas above basal, insulin concentrations were higher ($p<0.01$) after the mixed meal than the OGTT in both the IFG (62.9±4.9 vs. 42.0±4.3 nmol/4 hrs) and NFG groups (50.2±6.2 vs. 23.8±2.6 nmol/4 hrs) indicating more insulin was required to achieve the same postprandial glycemic response in the presence of non-glucose nutrients. On the other hand, C-peptide concentrations also were higher ($p<0.05$) after the mixed meal than the OGTT in the IFG (384±22 vs. 296±22 nmol/4 hrs) and NFG (355±40 vs. 238±22 nmol/4 hrs) groups indicating that nutrients caused a compensatory increase in insulin secretion in both groups. Of particular interest, the nutrient induced delta (meal minus OGTT) in plasma insulin (21±3 vs. 26±7 nmol/4 hrs) and C-peptide (88±18 vs. 119±33 pmol/4 hrs) concentrations

did not differ in the IFG and NFG groups respectively. Similarly, while fasting plasma glucagon concentrations tended to be higher (p=0.06) in the IFG than NFG group (75±6 vs. 59±5 pg/ml), glucagon concentrations were comparably suppressed during the OGTT (−3197±601 vs. −2385±1160 pmol/4 hrs) and if anything increased less (p<0.05) in the IFG than NFG group after mixed meal ingestion (1162±763 vs. 4925±1446 pmol/4 hrs) resulting in a comparable nutrient induced delta in glucagon (4359±842 vs. 7310±2099 pmol/4 hrs) in the IFG and NFG groups.

Conclusion: When the same amount of glucose is ingested a) more insulin is required to achieve a comparable postprandial glycemic response in the presence than in the absence of non-glucose nutrients, b) this increase in insulin requirement is compensated by a nutrient induced increase in insulin secretion in both IFG and NFG, and c) although glucose induced insulin secretion is impaired in IFG, the ability of nutrients to enhance glucose induced insulin secretion and to modulate postprandial glucagon release is intact.

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Association of neurogenin 3 polymorphisms with insulin secretion

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Background and Aims: Mutations in the transcription factors which play a role in the development of endocrine pancreas are associated with diabetes mellitus (DM). Neurogenin 3 belongs to a group of cell specific transcription factors which are expressed during embryogenesis. In mice with functionless Neurogenin 3, the endocrine pancreas is not developed and these mice die of diabetes early in postnatal period. In human, rare mutations in this gene are associated with MODY (maturity diabetes of the young). Inconsistent results of association studies dealing with the relation of *Neurogenin 3* polymorphisms to insulin secretion in patients with common forms of diabetes mellitus type 2 (DM2) are published.

Materials and Methods: DM2 (n=246; age 58,81±6,98; BMI 25,85±4,14 kg/m^2), offspring of DM2 (n=120; age 34,15±10,93; BMI 24,56±5,02 kg/m^2), healthy controls - non-diabetic subjects without family history of DM2 (n=238; age 32,23±10,67; BMI 23,21±3,67 kg/m^2), women with gestational diabetes (n=112; age 31,6±4,6; BMI 24,7±4,0 kg/m^2) entered the study. All subjects were anthropometrically and biochemically well characterized. In groups of controls and offspring, the oral glucose tolerance test (oGTT) and insulin tolerance test (ivITT) were performed and more than 20 indices of insulin secretion and insulin sensitivity were calculated. *Neurogenin 3* gene polymorphisms were identified by SSCP method and the results were confirmed in all subjects by RFLP (enzyme SmaI - Gly167Arg; enzyme Eam1104I - Ser199Phe).

Statistical methods: Chi-Square test, ANCOVA (adjustments for age and BMI). Anthropometric parameters were evaluated for men and women separately.

Results:

Genotype	DM2	Offspring of DM2	Controls	Gestational diabetics	p
Gly167Arg					
Gly/Gly	89.55%	93.60%	91.67%	90.63%	n.s.
Gly/Arg	8.96%	4.80%	6.77%	9.38%	
Arg/Arg	1.49%	1.60%	1.56%	0%	
Ser199Phe					
Ser/Ser	41.53%	48.97%	40.50%	45.30%	n.s.
Ser/Phe	44.76%	41.58%	43.73%	47.01%	
Phe/Phe	13.71%	9.45%	15.77%	7.69%	

We did not find any significant differencies in the allelic and genotypic frequencies of two screened *Neurogenin 3* polymorphisms among our study groups. However, statistical analysis revealed significantly lower basal as well as stimulated C-peptide levels in Phe allele carriers in comparison to Ser homozygotes (Ser199Phe).

Conclusion: We suggested that *Neurogenin* 3 variants could contribute to the deterioration of beta-cell secretion and thus influence the risk of DM2 development.

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Glucolipotoxicity severely damage insulin secretion and cause ketone overproduction

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Background and Aims: We have found in a previous study that obese diabetic patients with unprovoked ketosis have severely insulin resistance and markedly impaired β-cell insulin secretion. To explore the roles of elevated glucose and FFAs in the pathogenesis of ketone overproduction in obese diabetes, here we test the effect of FFAs in

the presence or absence of elevated glucose on insulin secretion and ketone production.

Materials and Methods: Diet-induced obese Wistar rats were assigned to three groups and all underwent 48 hr iv infusions in each group: Controls (NS, n=11): saline alone; FFA (n=11): 20% intralipid+heparin, 0.5 ml/h to maintain circulating FFA concentration at 1000–2000 μM; GS-FFA group(n=12): 25%glucose+20% intralipid+heparin in amounts required to maintain circulating glucose and FFAs concentration at 15–20 mM and 1000–2000 μM respectively. Serum FFA and β-hydroxybutyrate (β-HBA) were measured before and after infusion. Ten minutes after the end of the infusion, an IVGTT was performed in each group to evaluate the glucose-stimulated insulin secretion (GSIS) *in vivo*. The isolated rat pancreases preparations were applied to test the GSIS *in vitro*. Pancreases were perfused with Krebs-Ringer bicarbonate buffer contained glucose (16.7 mM) or arginine (19 mM) respectively.

Results: Serumβ-HBA levels, which were similar at baseline in various groups, were markedly elevated at the end of infusion in GS-FFA group (NS: 0.290±0.170 mM, FFA: 0.526±0.107 mM, GS-FFA: 1.257±0.344 mM, p value was less than 0.05). The glucose/insulin ratio during IVGTT was significantly increased in GS-FFA group (Table 1, p value was less than 0.05). Insulin secretions from perfused pancreases were similar in each group during the basal period (3 mM Glucose). The glucose and arginine stimulated insulin secretions were obviously declined in GS-FFA group when compared with FFA and NS group (Table 2, p value was less than 0.01).

Conclusion: A marked synergistic effect of high glucose and FFAs on impaired insulin secretions and ketone overproduction was demonstrated in this study. It is suggested that the pathogenesis of ketone overproduction in obese diabetes may be associated with dramatically declined β-cell insulin secretion, as a result of synergistic effect of high glucose and FFAs. The insulin deficiency results in long-chain Fatty acyl CoA accumulated in cytosol of hepatocyte transport into mitochondrion in large amount and cause ketone overproduction.

Table 1. Serum glucose/insulin concentration during IVGTT in three groups

Time (min)	NS group	FFA group	GS-FFA group
0	5.7±0.6/31.3±2.4	5.5±0.3/31.2±4.1	7.3±0.3/34.3±7.4
5	14.3±1.3/135.4±11.2	14.6±1.2/129.1±10.1	17.2±1.1*/65.3±4.5*
10	11.3±0.9/112.6±5.3	12.4±0.8/107.1±6.1	14.8±0.8*/75.6±5.2*
15	9.1±0.8/94.8±10.3	11.0±0.6/87.3±6.6	13.2±1.1*/66.4±4.7*
20	8.3±0.4/70.0±5.4	9.3±0.9/64.7±1.9	11.2±0.8*/56.8±5.9*

Table 2. Glucose and arginine stimulated insulin secretion from perfused pancreases (μU/min)

	NS group	FFA group	GS-FFA group
Baseline	123.4±52.9	122.3±45.9	106.7±39.6
Glucose (16.7 mM)	386.7±64.8	356.4±47.1	158.3±48.1*
Arginine (19 mM)	601.1±106.2	548.5±64.3	233.1±61.6*

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0634

The functional maturation of insulin secretion during late stage of pancreatic development in rat

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Background and Aims: Defect of insulin synthesis, secretion and sensitivity in target cells plays an important role in the pathogenesis of diabetes mellitus. Insulin exocytosis is essential for blood insulin level, though relatively less studied. According to the hypothesis of thrifty phenotype, it is considered that adult diabetes has a close relation to abnormal embryonic development of pancreas. Therefore it is important to study the pancreatic development and pancreatic gene expression among this complex process. We have investigated gene expression profiles during various stages of embryonic pancreatic development of rats in our previous work. The aim of the present study is to define key genes relevant to insulin synthesis and exocytosis, and to investigate the improved process of insulin secretion.

Materials and Methods: Pancreata of SD rat at embryonic day 12.5(E12.5),E15.5,E18.5 and newborn,21 days after birth,adult rats were dissected respectively. Genechips from Affymetrix company were applied to explore gene expression profiles. Some genes related to insulin synthesis and secretion were verified by Realtime PCR. Intraperitoneal glucose tolerance tests (IPGTT) and ELISA were done to detect the function of pancreatic islet.

Results: Among genes related to insulin synthesis and release, insulin,proinsulin and relevant transcription factors such as PDX-1 began to be expressed from E12.5; Munc13-1,syntaxin-1a,Rab3a,Vamp-2,Glut-2 began to be expressed from E15.5, while GCK was expressed at late stage of embryonic day. The result of IPGTT revealed that newborn rats before a week after birth were significantly

glucose intolerant showing an increased excursion in blood glucose in response to a glucose challenge. Basic blood insulin level was detected at E18.5 and increased rapidly thereafter. However, in response to a glucose load, only rats older than a week after birth began to show normal insulin secretory response gradually.
Conclusion: Potential ability of insulin synthesis and release in rat occurs from early and middle stage of pancreatic development. But the normal insulin secretion response to glucose challenge starts later.
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0635

Beta cell glucolipotoxicity induced by a diet high in saturated fat during pregnancy is reversed by PPARalpha activation
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Background and Aims: Glucose is the major physiological regulator of insulin secretion; however, chronically elevated levels of glucose contribute to worsening beta cell function in type 2 diabetes (glucotoxicity), particularly when in combination with elevated lipids (glucolipotoxicity). Pregnancy causes increased glucose transport into beta cells, and a decreased glucose threshold for glucose-stimulated insulin secretion (GSIS); hence adverse effects of chronic hyperglycemia on beta cell function may be exacerbated in pregnancy, particularly as late pregnancy is also associated with hyperlipidemia.
Materials and Methods: Pregnant rats were maintained on either a control (low-fat) diet or a high-saturated-fat diet from the onset of pregnancy and sampled at day 19 of pregnancy (term=22–23 days). The PPARalpha agonist WY14,643 was administered (50 mg/kg body wt.; i.p.) at day 18 of pregnancy (i.e. 24 h before sampling). Glucose-stimulated insulin secretion (GSIS) was assessed *in vivo* after acute glucose bolus injection, and *in vitro* using step-up and step-down islet perifusions
Results: Maintenance of rats on a high-saturated fat diet throughout pregnancy caused post-absorptive hyperglycemia (by 26%) and glucose intolerance at day 19 of pregnancy (term=23 days), with an elevated ΔG_{0-30} (38%; $P<0.05$) and decreased glucose disappearance rates by 41%; $P<0.001$) after intravenous glucose challenge. Although GSIS *in vivo* was increased (a 59% increase in ΔI_{0-30} following glucose challenge; $P<0.05$), isolated islets showed markedly impaired GSIS (a 45% decline in total insulin secretion; $P<0.01$) during step-up and step-down islet perifusionss, with an elevated glucose threshold for GSIS as well as decreased glucose responsiveness. To test whether impaired GSIS ex vivo reflected glucolipotoxicity, pregnant rats maintained on high-saturated fat diet were treated for 24 h with the PPARalpha agonist WY14,643 to augment lipid clearance via oxidation. This manipulation reversed fasting hyperglycemia. Although glucose tolerance remained impaired, GSIS with islet perifusions was significantly improved (by 63%; $P<0.05$), with complete restoration of glucose responsiveness and a substantial lowering of the glucose threshold for GSIS.
Conclusion: It is concluded that beta-cell glucolipotoxicity is induced by a diet high in saturated fat through increased lipid delivery, in the absence of a corresponding increase in lipid oxidation, in combination with its consequence, chronic fasting hyperglycemia.
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PS 44

Human liver metabolism

0636

Elevated plasma free fatty acids exacerbate underlying hepatic insulin resistance in type 2 diabetes
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Background and Aims: Elevated FFAs stimulate gluconeogenesis in people with type 2 diabetes mellitus (DM), however their effects on endogenous glucose production (EGP) are less certain due to autoregulation or presence of insulin concentrations that result in near maximal suppression of glycogenolysis.
Materials and Methods: Twentythree DM subjects and 13 matched non-diabetic controls (ND) were randomly studied twice. Intralipid-heparin (ILH) or glycerol (Gly) were infused for 11 hours. DM subjects were kept euglycemic during the night with a variable insulin infusion, thereafter insulin was infused in all subjects at rate of 0.6 mU/kg total body weight along with somatostatin, glucagon and growth hormone thereby maintaining equal portal concentrations. ^2H_2O was given the evening before for measuring gluconeogenesis; [3-^{3}H]glucose was infused to measure EGP; plasma glucose was clamped at 5.2 mM.
Results: Plasma FFA was higher ($p<0.01$) ILH vs Gly both before and during clamp in the DM (1.3±0.3 vs 0.3±0.0; 1.3±0.3 vs 0.3±0.1 mmol/L respectively) and ND (0.9±0.4 vs 0.1±0.0; 0.4±0.0 vs 0.0±0.0 mmol/L respectively).

Plasma glucose (5.2 mM), insulin (180 pmol/L), and C-peptide (0.02 ng/mL) did not differ ILH vs. Gly or between groups. Less ($p<0.01$) glucose was required to maintain euglycemia during ILH than Gly both in DM and ND (9.2±2.2 vs 16.5±2.9 and 32.6±4.5 vs 42.4±3.7 mol/kg/min). In DM this was due to both lower ($p<0.001$) glucose utilization (16.0±1.5 vs 22.1±2.5 mol/kg/min) and higher ($p<0.05$) EGP (6.8±0.8 vs 5.7±0.7 mol/kg/min). In ND, whereas glucose utilization was lower ($p<0.001$) on ILH vs Gly (37.2±3.7 vs 45.3±3.8 mol/kg/min), EGP did not differ (3.6±1.2 and 2.8±1.0 mol/kg/min). Despite lower ($p<0.01$) plasma FFA, EGP was higher ($p<0.05$) during the clamp in DM on Gly day vs.ND on ILH day implying an additional abnormality in hepatic insulin action.

Conclusion: People with type 2 diabetes have hepatic insulin resistance that is further exacerbated by elevated plasma FFA.

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0637

Liver fat accumulation and isolated islets function in non-diabetic and type 2 diabetic subjects

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Background and Aims: Overall adiposity, and visceral fat (VF) tissue accumulation in particular, is associated with insulin resistance and type 2 diabetes. However, the specific role of VF in beta-cell function is still unclear.

Materials and Methods: We determined liver fat (LF) accumulation (as a definite measure of VF) by histology and assessed isolated pancreatic islets insulin secretion and survival of 45 non-diabetic (ND; age: 61±14 yrs; M/F: 27/18; BMI: 24.5±2.7 Kg/m^2; waist circumference: 93.0±12.7 cm) and 12 type 2 diabetic (T2DM; age: 68±7 yrs; M/F: 7/5; BMI 27.6±2.6 Kg/m^2, $p<0.01$ vs ND; waist circumference 103.2±8.9 cm, $p<0.01$ vs ND) multi-organ donors.

Results: Average fat infiltration tended to be higher in T2DM (10.9±3.1%, range 0 to 35%) than in ND (6.2±8.9%, range 0 to 50%), and subjects with>20% LF were 25% in T2DM and 9% in ND. Correlation of LF was strong with waist circumference ($p<0.001$), weak with BMI ($p<0.05$) and absent with age. Isolated islets insulin release (IR, μU/islet/min) was 0.036±0.013 in ND and 0.033±0.008 in T2DM in response to 3.3 mmol/l glucose (G), and 0.076±0.057 in ND ($p<0.01$ vs 3.3 mmol/l G) and 0.044±0.021 in T2DM (NS vs 3.3 mmol/l G and $p<0.05$ vs ND) in response to 16.7 mmol/l G. Apoptotic rate, as assessed by determination of cytoplasmic histone-associated DNA fragments, was 0.57±0.54 and 1.17±0.87 optical density units in ND and T2DM islets, respectively ($p<0.05$). In ND, glucose-stimulated IR and apoptotic rate did not correlate with LF ($R=0.1$, $p=0.5$ and $R=0.1$, $p=0.6$, respectively); in T2DM, insulin release tended to be inversely correlated to LF ($R=0.5$, $p=0.06$).

Conclusion: We found therefore that histologically determined LF amount tended to be higher in T2DM and correlated mainly with waist circumference; however, ex-vivo pancreatic islet experiments failed to show strong associations between glucose-stimulated IR or islet cell apoptosis with LF, suggesting that if such a correlation occurs in- vivo, this is probably due to circulating factors.

0638

Noninvasive analysis of hepatic gluconeogenic and Krebs cycle fluxes in type 1a glycogen storage disease

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Background and Aims: In Type 1a glycogen storage disease (GSD), G6P-ase activity is absent resulting in no glucose or net glycogen synthesis from gluconeogenic precursors. To determine the effects of this block on hepatic gluconeogenic and Krebs cycle fluxes, GSD patients and healthy controls were given oral [U-^{13}C]glycerol, Paracetamol and phenylbutyric acid. On entry of [U-^{13}C]glycerol into hepatic Triose-P, UDPglucose ^{13}C-enrichment can be noninvasively analyzed via urinary Paracetamol glucuronide (PG). Likewise, ^{13}C-enrichment of Krebs cycle metabolites can be measured via phenylacetylglutamine (PAGN).

Materials and Methods: Five GSD patients (3M, 2F; 14±6 yr; 35±9 kg) and 5 controls (1M, 4F; 20±4 yr; 53±13 kg) were studied. At 08:00, 11:00 and 14:00, subjects ingested 1 g/kg body wt of cornstarch. Paracetamol (10–15 mg/kg) was ingested at 11:00 and phenylbutyric acid (5 mg/kg) plus [U-^{13}C]glycerol (25–30 mg/kg) at 14:00. Blood (10 ml) was collected at 17:00 and urine was collected from 16:45–18:45. Urinary PG and PAGN were isolated by ion-exchange chromatography and ^{13}C-enrichment and isotopomer distributions analyzed by ^{13}C NMR.

Relative metabolic fluxes by 13C isotopomer analysis: *p<0.05; **p<0.01 vs controls

Subject Group	Citrate synthase flux	Pyruvate dehydrogenase flux	Pyruvate carboxylase flux	Pyruvate kinase flux	Net anaplerotic flux	Plasma glucose from G6P-ase (%)	Triose-P to UDPG flux
Controls	1.0	0.3±0.1	9.9±3.3	8.7±2.8	1.3±0.5	56±13	100±31
GSD Patients	1.0	0.5±0.2**	16.6±3.5**	14.4±3.8*	2.2±0.8	not detected	8±8**

Plasma glucose was analyzed by ^{13}C NMR following deproteinization of plasma. The minimum fraction of plasma glucose derived via G6P-ase was estimated from the ratio of plasma glucose to PG ^{13}C-enrichment. Triose-P to glycogen flux in GSD patients compared to controls was estimated from relative PG ^{13}C-enrichment levels. Krebs cycle and pyruvate fluxes were estimated by analysis of PAGN ^{13}C NMR data.

Results: In controls, both plasma glucose and glucuronide were enriched with ^{13}C indicating active hepatic glucose production. At least half of the plasma glucose was derived via G6P-ase (Table 1). GSD patients had no glucose ^{13}C-enrichment and minimal PG enrichment indicating no G6P-ase flux and little flux into glycogen. In GSD patients, pyruvate oxidation, carboxylation and recycling were all significantly elevated compared to controls. Surprisingly, net anaplerotic fluxes tended to be higher despite the blockage of gluconeogenesis, the principal anaplerotic outflow pathway in liver. Plasma lactate was enriched in GSD patients despite the absence of ^{13}C-glucose. Isotopomer analysis indicates that the ^{13}C-lactate was formed via the anaplerotic pathways of the hepatic Krebs cycle rather than by glycolysis.

Conclusion: In GSD, there was no gluconeogenic outflow to glucose and glycogen but pyruvate utilization by the Krebs cycle was increased. There was a redirection of anaplerotic outflow from gluconeogenesis to lactate production. Unlike glycolysis, lactate synthesis from anaplerotic precursors does not generate metabolic acidosis, hence this route may allow the safe production of lactate as an alternative oxidizable substrate to glucose for use by peripheral tissues.

0639

Quantifying direct and indirect pathways of hepatic glycogen synthesis with deuterated water and paracetamol

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Background: Hepatic glycogen is synthesized by direct and indirect pathways (Fig. 1). Normally, the majority of glycogen is synthesized by direct pathway, but in Type 1 diabetes (T1D) patients, the indirect pathway predominates. The indirect pathway has futile metabolic cycles while the direct pathway is energetically more efficient. The direct pathway is further facilitated by the association of glucokinase with the glycogen synthesis enzymes - a process that is stimulated by the presence of insulin. In T1D patients, intensive insulin therapy does not restore direct pathway activity to normal levels. Novel interventions that target insulin delivery to hepatocytes, such as islet transplantation, may prove more effective. To effectively address such questions, we present a highly practical method for quantifying direct and indirect pathway activities in the clinical setting.

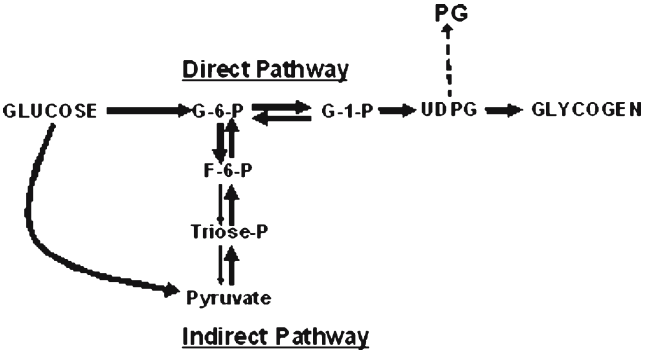

Figure 1. Direct and indirect pathways of hepatic glycogen synthesis and the sampling of UDPG as Paracetamol glucuronide (PG). In the presence of ^2H_2O, glucuronide formed by direct pathway is labeled in position 2 as a result of G6P-F6P exchange. Glucuronide synthesized by indirect pathway is labeled in both positions 2 and 5 as a result of triose-P isomerase and other exchange reactions.

Methods: 9 healthy subjects (3F/6M, 21±1 yr, 67±4 kg, Hb_{1Ac} 4.9±0.2%) and 8 T1D subjects (3F/5M, 20±1 yr, 65±3 kg, Hb_{1Ac} 8.1±0.6%) were studied. Subjects began fasting at 20:00. Paracetamol (1000 mg) and ^2H_2O (3.0 g/kg body-water) were taken orally at 02:00. At 08:00, subjects had breakfast (720 kcal, 60% CHO, 20% fat, 20% protein) and 500 mg Paracetamol. Urine was collected at

10:00–12:00 and ^{2}H-enrichment of PG and body water was quantified by ^{2}H NMR. Indirect pathway contribution (%) was estimated from glucuronide hydrogen 5 enrichment divided by that of body water (G5/bw)×100 and percent direct pathway contribution was estimated as 100 - indirect contribution. The percent of glucosyl units that did not undergo G6P-F6P exchange during glycogen synthesis, a putative marker for targeted glycogen synthesis via glucokinase, was estimated as 100×[1-(G2/bw)] where G2 is the glucuronide hydrogen 2 enrichment. Data are reported as means±SE.

Results: Body water enrichment was 0.27±0.01% for controls and 0.27±0.01% for T1D patients and was constant between 08:00 and 12:00 for both groups. G5/bw was significantly higher in TID patients compared to controls (0.53±0.02 *vs.* 0.41±0.02, p<0.03), indicating that a significantly smaller fraction of glycogen had been synthesized by the direct pathway (47±2% *versus* 59±2%, p<0.03). For both groups, glucuronide hydrogen 2 enrichment was significantly lower than that of body water (0.21±0.01 for controls; $p<10^{-4}$ and 0.22±0.01 for T1D patients, p<0.01). This indicates that a substantial portion of glucosyl units did not undergo G6P-F6P exchange during conversion to glycogen (22±3% for controls and 18±5% for T1D patients).

Conclusion: A simple and noninvasive method for quantifying the pathways of hepatic glycogen synthesis in humans was demonstrated. The measurement revealed significant differences in the direct pathway contribution to glycogen synthesis between healthy and T1D subjects.

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0640

Indices of insulin sensitivity of glucose production and disposal from double-tracer oral glucose tolerance test

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Background and Aims: The oral glucose tolerance test (OGTT), used with only two glucose concentration measurements for the diagnosis of glucose intolerance and diabetes, is increasingly employed as dynamic test in more complex experiments for the study of metabolic disorders. In fact, in addition to its simplicity of execution, OGTT elicits a physiological secretory response from splancnic organs of glucose regulating hormones including gastrointestinal incretins. Aim of this study was the development of a simplified method for the analysis of data from double-tracer OGTT experiments, which are used for reconstructing rates of absorption, elimination and hepatic production (HGP) of glucose.

Materials and Methods: The proposed approach is based on a new mathematical model, derived from the minimal model of glucose disappearance proposed by RN Bergman and C Cobelli, which quantifies univocally the disappearance of both cold and tracer glucose by means of glucose clearance at zero insulin (GEZI) (dl/min) and insulin sensitivity of glucose disappearance S_I (dl/min/(μU/ml)). The novelties consist in the description of: (i) inhibition of HGP by insulin through a parameter, I_{50} (μU/ml), which quantifies the deviation of insulin concentration from basal required to inhibit HGP by 50%; and (ii) gastrointestinal glucose absorption as a function of only two parameters. Model parameter estimates were obtained by means of the statistical non-linear mixed effects population approach. Twelve type 2 diabetic patients (D) (7 males and 5 females, age=53.6±2.5 yrs, BW=81.9±3.6 kg, BMI=30.5±1.1 kg m^{-2}) and 10 control subjects with normal glucose tolerance (NGT) and similar BMI (5 M and 5 F, age=39.6±3.7, BW=90.1±3.9, BMI=31.1±0.9), underwent a double-tracer OGTT with primed-continuous infusion of 0.25 μCi/min [3-^{3}H]-glucose starting at T=−180 min in D and −120 in NGT, and with oral load at T=0 of 75 g glucose dissolved in water together with 75 μCi [1-^{14}C]-glucose. Blood samples for measurement of concentrations of cold and tracer glucose and insulin were collected every 15 minutes between T=−30 and 240 min.

Results: Model parameter estimates indicate a population average glucose clearance at zero insulin independent of the diabetic state and gender with GEZI=0.72 dl/min (9.1% CV) at a reference BW of 85 kg that increases with BW by +2.1%/kg (0.7% CV); an insulin sensitivity of glucose disappearance in NGT subjects S_I(NGT)=0.098 dl/min/(μU/ml) (15% CV) about 20 times higher than in diabetic patients S_I(D)=0.0048 (18% CV); and an inhibition of HGP already at small suprabasal insulin levels I_{50}=16 μU/ml (24%CV), which was not significantly dependent on the diagnosis or gender.

Conclusion: This study suggests that type 2 diabetes is characterized predominantly by a reduction of insulin sensitivity of glucose disappearance, and supports the usefulness of a simplified mathematical modelling approach to assess glucose absorption, production and disposition during double-tracer OGTT experiments. The population analysis allows the assessment of factors, such as BW and diagnosis, that influence between-subject variability of these processes, and provides a means for planning additional experiments for validating the present results and for designing simplified protocols for a broader use in clinical studies.

0641

High hepatic stearoyl-CoA desaturase-1 (SCD-1) index is associated with low liver fat and high insulin sensitivity in obese humans

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Background and Aims: SCD-1 catalyzes the synthesis of monounsaturated fatty acids (FA) from saturated FA. It is imperative for the assembly of very low density lipoprotein (VLDL) particles, which transport FA from liver to adipose tissue and other sites. Studies in animals suggest that SCD-1 deficiency is beneficial for metabolism. However, in humans data are conflicting.

Materials and Methods: We applied an elaborate method to measure an index of hepatic SCD-1 activity. First, we determined the pattern of FA in the triglyceride fraction of the VLDLs by ultracentrifugation followed by thin layer separation and gas chromatographic quantification in plasma samples from 54 healthy subjects. The oleate (C18:1, n9)/stearate (C18:0) ratio was used as an index of hepatic SCD-1 activity, thereafter. Insulin sensitivity (IS) was estimated from an oral glucose tolerance test (OGTT) and determined during a euglycemic clamp (n=48). Liver fat was measured by 1H magnetic resonance spectroscopy.

Results: Gender distribution, mean age and body fat were as follows: 26 m/28 f, 44±2 y, 31±1%. The hepatic SCD-1 activity index was negatively associated with liver fat ($r=-0.28$, $p=0.049$), independently of age, gender and body fat. It was positively associated with IS(OGTT) ($r=0.42$, $p=0.002$) and weakly with IS(clamp) ($r=0.27$, $p=0.07$). Importantly, these relationships were dependent on overall adiposity. When subjects were divided in a lean (n=27, 26±5%) and in an obese (n=27, 35±6%) group by the median body fat, the significant relationships were absent in the lean group (liver fat: $p=0.34$, IS(OGTT): $p=0.75$, IS(clamp): $p=0.24$). However, they were strengthened in the obese group ($p=0.004$, $p=0.0002$, $p=0.006$).

After a 9 month lifestyle intervention with diet and increase in physical activity, a high SCD-1 activity index at baseline was associated with a larger increase in IS (clamp, adjusted) in the obese ($p=0.04$) but not in the lean ($p=0.35$) group.

Conclusion: We conclude that hepatic SCD-1 is an important regulator of liver fat and insulin sensitivity in humans. In addition, this interaction is modulated by adiposity.

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0642

Loss of hepatic clearance of insulin with resulting hyperinsulinaemia after orthotopic liver transplantation in humans

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Background and Aims: The regulation of carbohydrate metabolism is presumed to be restored by Orthotopic liver transplantation (OLT) in liver disease patients, but is likely to be affected by immunosuppression and the possible effects of hepatic denervation. The aim of our study was to evaluate regulation of hepatic glucose production following OLT.

Materials and Methods: To assess this further, we performed hyperinsulinemic, euglycemic clamp studies for 2 hours in 10 patients (47–66 yrs, BMI 19–38) who had undergone OLT 5 to 12 months earlier, and in 4 patients (32–57 yrs, BMI 27–33) who had undergone renal transplantation (RT) 3–6 months earlier and were on the same immunosuppression regimens as the OLT patients, and in 6 normal (NL) subjects (19–25 yrs, BMI 20–24). A primed-constant infusion of 3-^{3}H-glucose was begun 2.5 hr before the start of the clamp and continued throughout the hyperinsulinemic clamp. Insulin (10 mU/m^2/min) was infused from 0–120 min and glucagon (3.5 ng/kg/min) was infused from 60–120 min. The 20% glucose solution used to maintain euglycemia was spiked with ^{3}H-glucose (hot G_{inf}). Rates of hepatic glucose appearance (Ra, mg/kg/min), of overall glucose disappearance (Rd, mg/kg/min), and insulin levels (IRI, pM) were measured every 10 minutes from −30 to 120 minutes.

Results:

		BASAL	0–60 min	60–120 min
OLT	Ra	2.0±0.16	0.8±0.23	2.9±0.31
	Rd	2.0±0.16	2.5±0.23 †	2.4±0.31 †
	IRI	154+28 †	316±44 †	479±57 †
RT	Ra	2.4±0.45	2.0±0.30 †	2.2±0.14
	Rd	2.4±0.45	3.1±0.20	2.2±0.15 †
	IRI	48+17	115±23	120±27
NL	Ra	2.2±0.17	1.3±0.51	2.9±1.13
	Rd	2.2±0.20	3.1+0.31	3.7±0.64
	IRI	35±7	133±11	135±12

Mean values±SEM are shown († $p<0.05$ vs NL by t-test).

Hepatic glucose production after OLT is normal despite striking hyperinsulinemia. Basal C-peptide levels are elevated in OLT (3.3 ng/ml vs. 1.6 in RT and 1.5 in NL), but hepatic clearance of insulin is profoundly impaired in OLT (metabolic clearance rate of insulin: 36±4.3 ml/m^2/min) compared to NL (87±1.9) and RT (75±0.9). Hepatic sensitivity to insulin is decreased after both OLT and RT, which suggests a drug effect, but peripheral sensitivity to insulin is impaired only after OLT.

Conclusion: 1) OLT results in significant impairments in hepatic sensitivity to, and clearance of, insulin; 2) systemic insulin levels increase 3–4 fold largely due to a decrease in hepatic clearance of insulin; 3) peripheral insulin sensitivity is down-regulated by the hyperinsulinemic state; and 4) the loss of hepatic clearance of insulin appears independent of drug effects, and is probably secondary to interruption of CNS regulation of hepatic glucoregulatory functions.

Supported by NIH

PS 45

Liver metabolism - animal models

0643

AMPK regulates HNF4alpha transcription by the inhibition of Sp1 activity

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Background and Aims: The transcription factor hepatic nuclear factor 4α (HNF4α) is a regulator of the expression of genes involved in energy metabolism in the liver, intestine, and endocrine pancreas. It has been shown recently to play an important role in the regulation of hepatic glucose output, a key component of the maintenance of plasma glucose levels. Previous study reported that HNF4α is a downstream target of AMPK and that AMPK activation is decreasing protein stability of HNF4α. It has been also studied that Sp1 binds to the promoter region of HNF4α. In this study we investigated whether the regulation of Sp1 by AMPK could affect HNF4α expression.

Materials and Methods: AMPK was overexpressed with adenoviral -mediated AMPK gene(Ad.AMPK) in HepG2 cells for 2 days after virus infection. Adenoviral -mediated AMPK -kinase dead gene(Ad.KD) was used for inhibition of AMPK kinase activity. To identify *in vivo,* SD rats were adminstered intravenously with Ad.AMPK and Ad.lacZ as control. The activation of AMPK was confirmed by Western blot analysis with HepG2 lysates and extracts of liver tissues. The expression levels of HNF4α were evaluated with anti-HNF4α antibody. The phosphorylation of Sp1 by AMPK was determined by *in vitro* kinase assay or with anti-phospho-serine antibody.

Results: Activated AMPK by Ad.AMPK infection decreased protein level of HNF4α. Protein level of HNF4α was not decreased in Ad.KD infected cells or Ad.LacZ injected rats. Phosphorylation of Sp1 was increased in Ad.AMPK infected HepG2 cells. Ad.KD infection or treatment with AMPK inhibitor did not show the phosphorlyation of Sp1. Phosphorylation of Sp1 inhibited the transcription of HNF4α. These results were confirmed by decreased protein levels of HNF4α in Ad.AMPK administrated SD rats.

Conclusion: Activated AMPK by Ad.AMPK increased Sp1 phosphorylation and decreased protein levels of HNF4α. Sp1 phosphorylation may play an inhibitory role in transcription of HNF4α gene. Conclusively, overexpression of AMPK may down-regulate HNF4α transcription by inhibitory Sp1 phosphorylation. Down-regulated HNF4α by AMPK activation may diminish the interaction of HNF4α with PGC-1and may suppress gene expression related to gluconeogenesis.

0644

Adenoviral-mediated AMPK gene expression ameliorates hyperglycaemia and hyperlipidaemia in overt type 2 diabetic rats

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Background and Aims: AMP-activated protein kinase (AMPK) has been proposed to regulate the activities of key enzymes of lipid synthesis and metabolism and to improve insulin sensitivity. The Otsuka Long-Evans Tokushima Fatty (OLETF) rat is an established animal model of human type 2 diabetes that exhibits chronic and slowly progressive hyperglycemia and hyperlipidemia and is accompanied by progressive fibrosis in the islets. We have investigated the effects of AMPK in overt type 2 diabetic rats via adenoviral-mediated AMPK gene expression.

Materials and Methods: 42 weeks of aged overt type 2 diabetic rats (n=10) were administered intravenously with adenoviral-mediated AMPK gene (Ad.AMPK). Adenoviral-mediated lacZ gene was used for control group (n=10). Expression of Ad.AMPK was confirmed by

RT-PCR and Western blot analysis. AMPK activity was measured by phosphorylation of acetyl CoA carboxlyase (ACC) as a substrate of AMPK. Blood samples were collected from the tail vein for measurement of insulin, glucose, triglyceride, cholesterol and free fatty acid just before injection of Ad.AMPK for vehicles and on the 3rd and 7th days after Ad.AMPK administration. To investigate the changes of gene expression related glucose and lipid metabolism, quantitative real-time PCR was performed with liver tissues.

Results: Administration of Ad.AMPK showed high expression of AMPK in liver for 1 week and then decreased to level of control rats. Activity of AMPK in Ad.AMPK injected rats confirmed by phosphorylation of ACC. For transiently high expressed period (usually 1 week), Ad.AMPK injected rats improved hyperglycemia and hyperlipidemia. Plasma concentration of metabolic parameters is presented on the attached table. However, circulating level of free fatty acid did not change significantly on 3 or 7 days in Ad.AMPK administrated rats. The changes of metabolic gene expression showed that gluconeogenesis-related genes, phosphoenol pyruvate carboxykinase (PEPCK) and G6Pase were down-regulated in Ad.AMPK injected rats, and that lipogenesis-related gene, fatty acid synthase (FAS), stearoyl-CoA desaturase-1 (SCD-1) and glycerol-3-phosphate acyltransferase (GPAT) were also decreased in Ad. AMPK injected rats.

Conclusion: Adenoviral-mediated AMPK gene was successfully expressed in the liver of Ad.AMPK injected rats and the expressed AMPK phosphorylated its substrate. The effects of AMPK were improvement of hyperglycemia and hyperlipidemia and suppression of gene expression related with gluconeogensis and lipogenesis. These results suggest that intravenous administration of Ad.AMPK may play important role in liver and the effects of AMPK be directed to metabolism of glucose and lipid. Even though the expression period of Ad.AMPK was so short that the effects of AMPK showed transiently, long-term expressed Ad.AMPK would be promising therapeutic modality for treatment of type 2 diabetes.

Plasma levels of metabolic parameters in diabetic rats 0, 3 and 7 days postinjection with Ad.AMPK

Parameter	0 day	3 day	7 day
Glucose (mg/dl)	291.5±45.0	257.4±56.3	212.6±34.3*
Insulin (ng/ml)	1.022±0.252	1.456±0.211	2.212±0.346*
Triglyceride (mg/dl)	182.0±35.9	104.7±25.4*	180±45.2
Total cholesterol (mg/dl)	399.2±78.3	246.9±45.2*	431±54.5
Free fatty acid (uEq/L)	454.8±87.3	389±56.9	488±77.6

0645

Profile of rhythmic gene expression in the liver of obese diabetic KK-A^y mice

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Background and Aims: In vivo effect of type 2 diabetes on gene expression is usually studied only at one scheduled time of day. However, such a result might be affected by an observation time, because the expression of many genes exhibits circadian, 24-h rhythmicity in various tissues, including liver. At present, it remains to be determined whether the influence of type 2 diabetes on the gene expression is greater than that of the circadian time. To address this issue, we investigated the effects of experiment time on hepatic mRNA levels of various genes in genetically obese, diabetic KK-A^y and control KK mice.

Materials and Methods: Female 8-week-old KK and KK-A^y mice (n=12 in each strain) were maintained under a 12-h light/12-h dark cycle for 2 weeks, and thereafter the liver samples were obtained at the following zeitgeber times (ZT): 0, 6, 12, and 18, where ZT 0 is defined as lights on and ZT 12 as lights off. Transcript levels of various genes in each sample were determined by microarray (GeneChip Mouse Expression Array 430A) and real-time quantitative PCR analysis.

Results: Severe obesity, obvious hyperglycemia and hyperinsulinemia were detected in KK-A^y but not in KK mice. A Gene tree clustering using the samples obtained from both strains at ZT 0 and 12 suggested the greater effects of the ZT on gene expression than those of obese diabetes as a whole. The real-time PCR of the genes whose microarray expression levels differed markedly (>5-fold, P<0.05) between ZT 0 and 12 in either strain revealed that 22 of 23 genes showed circadian expression rhythms even in KK-A^y mice. Obese diabetes did not affect the phases, but reduced the peak levels in half of the genes. Although the mRNA levels of most (16 of 22) genes significantly differed between KK and KK-A^y mice at one or more ZT, only 3 of these 16 genes showed the significant differences throughout the day.

Conclusion: Because obese diabetes alters the expression levels of various genes only at some observation time points, the consideration of expression rhythmicity could be critical in interpreting gene expression data.

0646

Undetectability of intestinal gluconeogenesis in fed and fasted rats: In vitro carbon 13 NMR spectroscopy and *in vivo* evidence

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Background and Aims: It is classically admitted that only the liver and the kidney have the capacity to synthesize glucose from non-carbohydrate precursors, mainly lactate, alanine, glutamine and glycerol. However, recent studies have reported that the small intestine of fasted and diabetic rats substantially contributes *in vivo* to systemic glucose production via gluconeogenesis. Given the potential pathophysiological importance of intestinal gluconeogenesis, we have re-examined this subject.
Materials and Methods: For this, metabolically viable cells prepared from the small intestine of fed and fasted adult Wistar rats were incubated in Krebs-Henseleit medium under well-oxygenated conditions in the presence of 5 mM [3-^{13}C]-glutamine without and with unlabelled glucose. At the end of incubation, substrate removal and product formation were measured by enzymatic and ^{13}C-NMR spectroscopy methods. Arterio-portal concentration difference measurements for substrate removal or production were also performed in 72 h-fasted rats.
Results: The intestinal cells from both fed and 72 h-fasted rats removed glutamine and synthesized glutamate, lactate, alanine, citrulline and $^{13}CO_2$ at high rates; despite the high rate of substrate removal, the synthesis of ^{13}C-labelled glucose remained undetectable both in the absence and the presence of unlabelled glucose during the incubation. By contrast, hepatic and renal cells isolated from fasted rats and incubated with 5 mM [3-^{13}C]-glutamine synthesized large amounts of glucose labelled on its carbons 1, 2, 5 and 6 to approximately the same extent. Similarly to what was observed with glutamine as substrate, no glucose synthesis was observed in intestinal cells isolated from 72 h-fasted rats and incubated with 5 mM glycerol or fructose despite a substantial rate of utilization of these substrates.
Arterio-portal concentration difference measurements for glucose, lactate, pyruvate, alanine, glutamine, glutamate, glycerol, ketone bodies and ammonia in anesthetized 72 h-fasted rats showed that the small intestine did not produce but rather removed glucose in net amounts. Only a small fraction of the circulating glutamine and glycerol, the precursors of the glucose reported to be synthesized *in vivo*, was taken up by the small intestine of these rats. Assuming the complete conversion of the latter fraction into glucose, the circulating radioactive glucose would be diluted by only approximaltely 1%. This means that, under these conditions, intestinal gluconeogenesis from glutamine and glycerol is not demonstrable *in vivo* because the hardly measurable dilution of circulating radioactive glucose would be smaller than the margin of errors of the methods used.
Conclusion: The existence of gluconeogenesis in the rat small intestine, which has been reported to explain up to 35% of endogenous glucose production in 72 h-fasted rats, is seriously challenged by the present results and, in our opinion, remains to be demonstrated.
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0647

Sustained activation of the mTOR nutrient sensing pathway associates with hepatic insulin resistance but not with steatosis *per se* in mice

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Background and Aims: Increased activation of nutrient sensing through mammalian traget of rapamycin (mTOR) has been linked to the pathogenesis of insulin resistance. We examined activation of mTOR-signaling in relation to insulin resistance and hepatic steatosis in mice.
Materials and Methods: Insulin resistance and chronic hepatic steatosis were induced by high-fat (HF) feeding of male C57/BL/Jico mice for 6 weeks. Acute hepatic steatosis in the absence of insulin resisance was induced by pharmacological blockade of b-oxidation at the level of carnitine palmitoyltransferase I using tatradecylglycidic acid. Mitochnondrial function and mTOR signaling were examined in liver homogenates.
Results: HF feeding caused increased body weight ($p<0.001$) and hepatic insulin resistance ($p<0.05$). The phosphorylation of mTOR and its downstream targets p70S6 kinase and S6 ribosomal protein were 2-fold higher in HF- versus chow-fed mice (all $p<0.05$), and associated with enhanced rates of protein synthesis. Acute induction of hepatic steatosis had no effect on mTOR activity. The increased activity of the mTOR pathway in livers from HF-fed mice could not be ascribed to diet-induced alterations in known modulators of mTOR activity, such as circulating plasma leucine levels, phosphor-

ylation of protein kinase B, AMP-activated protein kinase and changes in mitochondrial function.
Conclusion: HF-diet leading to insulin resistance, but not hepatic lipid accumulation *per se*, induces increased activation of the mTOR nutrient sensing pathway.
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0648

Characterisation of the human Ped-Pea/15 promoter: identification of a functional HNF4α response element

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Background and Aims: Type 2 diabetes is a heterogeneous disease caused by both environmental and genetic factors. The pathophysiology of type 2 diabetes results from insulin resistance coupled with functional defects in insulin secretion by pancreatic beta cells. However, the precise factors leading to this disease are not known.
PED/PEA-15 is a ubiquitously expressed multifunctional protein whose gene has been shown to be over-expressed in skeletal muscle and adipose tissue from individuals with type 2 diabetes. Studies in transgenic mice also indicate that over-expression of PED/PEA-15 causes insulin-resistance and impairs glucose tolerance. The aim of our study is to characterize the molecular mechanism involved in the regulation of Ped-Pea/15 gene expression.
Materials and Methods: We have investigated this issue in cultured Hela and HepG2 cells.
Results: We started our study by identifying the promoter region driving the transcription of the gene. A 4 Kb fragment of the 5′-flanking region of Ped-Pea15 was cloned in front of the luciferase gene. Transfection of the construct into Hela cells produced an increase in the luciferase activity. Deletion analysis of this construct led to the identification of a 288 bp fragment as a core promoter sufficient for the transcriptional activation of the gene. In Silico studies first indicated the presence of a consensus binding site for the Hepatocyte Nuclear Factor 4 alpha (HNF4alpha) between nucleotides −477 and −367, suggesting that HNF4alpha could be involved in the regulation of Ped-Pea-15 gene expression. Co-transfection experiments with the HNF4alpha expression vector led to a 60% reduction of the luciferase activity associated with Ped-Pea-15 promoter fragment carrying HNF4alpha binding site. This effect is specific, since it is lost when the cells were transfected with a 5′deletion construct lacking HNF4alpha site. As expected increasing concentrations of this nuclear receptor proportionally reduced the luciferase activity of the reporter gene. Consistent with these data Real Time PCR assay on total RNA extracted from HNF4alpha transfected Hela cells confirmed a decrease in Ped-Pea 15 expression level.
Finally, chromatin immunoprecipitation (ChIP) experiment conducted in HepG2 cells evidenced the presence of a functional HNF4alpha response element between nucleotides −477 to − 367 on the Ped-Pea/15 promoter.
Conclusion: Our results indicate that HNF4alpha downregulate Ped-Pea/15 mRNA expression through a direct or indirect action on HNF4alpha responsive element present on Ped-Pea/15 promoter sequence.

0649

PPAR-α activation upregulated liver X receptor-α and downregulated sterol regulatory element-binding protein-1c in liver of high-fat-fed and high-starch-fed rats

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Background and Aims: Recent data suggested that liver X receptor (LXR)-α played a dynamic role in the regulation of fatty acid metabolism. And sterol regulatory element-binding protein (SREBP)-1c activated transcription of the genes required for fatty acid synthesis in the liver. The present study was to examine the potential preventive effect of PPAR-α activition on the development of insulin resistance and on the expressions of both LXR-α and SREBP-1c in liver of high-fat-fed and high-starch-fed rats.
Materials and Methods: High-starch-fed and high-fat-fed rats were treated with PPAR-α activator fenofibrate for 8 weeks. Insulin sensitivity was evaluated using a hyperinsulemic-euglycemic clamp technique. The expressions of LXR-α and SREBP-1c were determined by real-time PCR and western blotting.
Results: Fenofibrate decreased body weight and visceral fat, but increased plasma adiponectin level. Furthermore, fenofibrate significantly decreased the triglyceride content in the liver and improved insulin sensitivity in both high-fat-fed and high-starch-fed rats. High fat feeding and high starch feeding stimulated the expression of SREBP-1c in liver. Fenofibrate induced the expression of LXR-α and decreased the level of SREBP-1c significantly.

Conclusion: The PPAR-α agonist prevented the development of insulin resistance and hepatic fat deposition in high-fat-fed and high-starch-fed rats, which might be related to the upregulation of LXR-α and downregulation of SREBP-1c in liver.

0650

Splanchnic and liver glucose metabolism: an insulin dose-response study

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Background and Aims: The present study was undertaken to investigate the response of splanchnic (pre-hepatic, SPL) and liver (LIV) glucose and gluconeogenic substrate fluxes to different levels of insulin.
Methods: Eighteen pigs underwent catheterization of the carotid artery, portal and hepatic veins for the measurement of lactate, pyruvate, alanine, beta-hydroxybutyrate, glycerol, glucose, free fatty acids and insulin. Blood flow was measured by Doppler flow probes. Animals were studied during fasting (n=10), 1 mU/kg/min (n=4) and 5 mU/kg/min (n=4) euglycemic hyperinsulinemic clamp. Glucose production (EGP) and gluconeogenesis (GNG) were assessed by stable tracer methods.
Results: In SPL, glucose uptake increased in proportion with insulin levels ($r=0.61$, $p=0.01$); hyperinsulinemia switched alanine and pyruvate balance from release to uptake ($p<0.05$) while no change was observed in the other substrates. In LIV, hyperinsulinemia diminished net glucose release by decreasing EGP by 60% during the low and 94% during the high insulin infusion ($p=0.05$ and 0.02 *vs* fasting, respectively); GNG% accounted for 80% of EGP at fasting, and was suppressed by>50% during the clamp ($p<0.03$). SPL insulin uptake went from release in the fasting state to uptake during the 1 mU-clamp, and was further doubled during the 5 mUclamp ($p<0.02$); LIV insulin uptake was increased by 7- and 45-fold from fasting to physiological and supraphysiological hyperinsulinemia, respectively ($p<0.0001$). The SPL uptake of insulin was inversely correlated with GNG ($r=-0.68$, $p=0.02$). When fluxes were calculated considering LIV and SPL as a whole compartment, release of GNG precursors and glucose uptake by the pre-hepatic SPL were fully masked, due to opposite higher LIV fluxes.
Conclusions: SPL substrate balance documented an insulin dose-dependent switch of GNG precursors from release to uptake, contributing to the suppression of GNG during hyperinsulinemia.
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0651

Novel mechanistical aspects in action of glycogen phosphorylase inhibitors on glycogen metabolism in primary rat hepatocytes

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Background and Aims: Hepatic glucose output is elevated in type 2 diabetes. Glycogen breakdown (glycogenolysis) contributes significantly to the abnormally high glucose production by the liver. Glycogen phosphorylase (GP) catalyzes glycogenolysis and several pharmacological agents inhibiting this enzyme have been described as potential anti-diabetic drugs attenuating hyperglycemia. Since glycogenolysis is in dynamic interplay with glycogen synthesis, it is important to explore the consequences of inhibiting GP for glycogen metabolism as a whole. Therefore, the aim of the study was to investigate the effects of different GP inhibitors on proteins involved in glycogen metabolism to better understand the mechanistic basis for their pharmacological profile.
Materials and Methods: Hepatocytes were isolated from the livers of male Wistar rats by collagenase digestion. The cells were maintained in William's E medium or DMEM supplemented with different glucose concentrations depending on the experimental design and for all studies treatment with compounds was performed 24 h after cell isolation. Glycogen synthesis was assessed by determination of [^{14}C]glucose incorporation into glycogen within 2 h at suitable glucose concentrations (e.g. 10 mM). Glycogen content was measured as glucose residues by amyloglucosidase method. To investigate the effects of GP inhibition on glycogen metabolism at the molecular level, glycogen-enriched fractions were prepared by differential centrifugation. Cellular distribution and phosphorylation status of proteins regulating glycogen metabolism were determined by immunoblotting.
Results: Administration of GP inhibitors not only inhibited GP activity but also stimulated glycogen synthesis using rat hepatocytes. This correlated with profound increase in glycogen levels (by 2 fold) upon application of the compounds at hyperglycemic conditions (20 mM glucose).

Compared to untreated controls, compound effects on glycogen amounts were even more pronounced (up to 5 fold increase) at conditions of complete glucose deprivation (0 mM glucose) suggesting prevention of glycogen store mobilization by GP inhibitors. With regard to proteins involved in glycogen metabolism, in cells treated with compounds at both high (10 mM) and low (2.5 mM) glucose, most of glycogen synthase (GS) associated with glycogen was in its less phosphorylated (more active) state as revealed by phospho(Ser641)-immunoblotting of glycogen-enriched fractions. Surprisingly, the amounts of glycogen-bound GP were dramatically reduced in compound-treated samples at high glucose. At low glucose this effect was somewhat blunted, albeit not abolished. Correspondingly, more GP was recovered in the cytosolic fractions obtained from compound-treated cells.

Conclusion: In the present study, GP inhibition increased glycogen levels in primary rat hepatocytes at both high and low glucose conditions suggesting increased energy pools - a protective measure to control diabetic hyperglycemia. Furthermore, the present study provides the evidence that, in addition to inhibiting GP enzymatic activity, cellular localization of GP and activation of GS are influenced. Thorough understanding of underlying mechanistic principles could help to design compounds with different pharmacological profiles.

0652

Estrogen improves glucose tolerance and hepatic insulin sensitivity in ob/ob mice

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Background and Aims: Recent studies have demonstrated that estrogen plays an important role in glucose homeostasis. This estrogen effect is mediated via estrogen receptor-α (ERα). Thus, in estrogen receptor-α knockout (ERKO) mice, impaired glucose homeostasis was observed which was mainly due to decreased hepatic insulin sensitivity resulting from the upregulation of lipogenic genes.

The aim of the present study is to investigate the mechanisms behind the effect of estrogen on glucose tolerance and hepatic insulin sensitivity in ob/ob mice.

Materials and Methods: ob/ob mice were treated with 17β- estradiol (100 μg/kg/day) for 30 days, while control ob/ob mice received vehicle. In both groups, we performed intraperitoneal glucose tolerance tests and insulin tolerance tests. Furthermore, genome-wide expression profiles were analyzed in the liver, using high-density oligonucleotide microarrays. Pathway analysis was performed to detect coordinated changes in the expression of groups of functionally related genes. Real-time PCR was used to confirm the expression of several genes which were found to be regulated by estradiol treatment.

Results: Estradiol treatment improved glucose and insulin tolerance and decreased basal blood glucose (8.0±0.48 vs 4.9±0.29 mM, $p<0.01$; control vs treated) and plasma insulin (204.6±19.1 vs 138.0±10.4 μU/ml, $p<0.01$; control vs treated) levels in ob/ob mice. Estradiol decreased the expression of gene categories involved in fatty acid and lipid biosynthesis. Among the genes involved in these categories, expression levels of fatty acid synthase, stearoyl-coenzymeA desaturase1, and glycerol-3-phosphate acyltransferase were significantly decreased. Further searches for genes directly targeted by estrogen revealed increased hepatic mRNA expression of signal transducer and activator of transcription 3 (Stat3). Furthermore, levels of Stat3 protein and phosphorylated Stat3 protein were increased in the liver of estradiol treated ob/ob mice. Finally, Stat3 expression was rapidly induced by estradiol in the mouse liver.

Conclusion: In conclusion, estradiol treatment improves glucose tolerance and insulin sensitivity in ob/ob mice. We propose that this may be mediated, at least partially, via estrogen stimulation of the hepatic expression of Stat3, leading to decreased expression of hepatic lipogenic genes and thereby to the observed antidiabetic effects.

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PS 46

Regulation of beta cell function by incretin hormones

0653

Sitagliptin, a dipeptidyl peptidase-4 inhibitor, improved β cell function in patients with type 2 diabetes: a model-based analysis

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Background and Aims: β-cell dysfunction occurs early in type 2 diabetes and is characterized by abnormalities such as depressed β-cell sensitivity to glucose with respect to insulin release. Incretins have been shown to enhance β-cell function in patients with type 2 diabetes and in animal models of

diabetes. Since sitagliptin increases active incretin levels, β-cell function was assessed using frequently sampled (9-points) meal tolerance tests (MTT) in sub-studies from 3 Phase III trials (2 monotherapy studies and an add-on to metformin study) using a model-based analysis.

Methods: For the MTT, blood was collected at −10, 0, 10, 20, 30, 60, 90, 120, and 180 min relative to start of the meal. Parameters for β-cell function were assessed using the C-peptide minimal model, which allows for the estimation of the insulin secretion rate and for characterization of the insulin secretion response into basal (Φ_b; β-cell responsiveness to basal glucose concentrations), static (Φ_s; β-cell responsiveness to above-basal glucose concentrations following a meal), and dynamic (Φ_d; β-cell responsiveness to the rate of increase in above-basal glucose concentrations following a meal) components. Insulin sensitivity was assessed with a validated composite index (ISI). The disposition indices, which assess insulin secretion in the context of changes in insulin sensitivity, were also assessed.

Results: When used as monotherapy or added to on-going metformin therapy, sitagliptin produced improvements in Φ_s and in Φ_b and overall responsiveness of the β-cell to glucose (Φ). The parameter describing dynamic sensitivity of the β-cell to glucose (Φ_d) showed numerical, but not statistically significant, improvements with sitagliptin. Disposition indices were also broadly improved with sitagliptin treatment relative to placebo. Despite enhanced β-cell sensitivity to glucose, the very low rate of hypoglycemia observed in clinical trials with sitagliptin indicated that the increase in β-cell function remained glucose-dependent.

Conclusions: In this model-based analysis, sitagliptin improved β-cell function in both fasting and postprandial states in patients with type 2 diabetes.

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0654

Differential effects of vildagliptin on islet function in patients with type 2 diabetes receiving no additional agent or concomitant metformin

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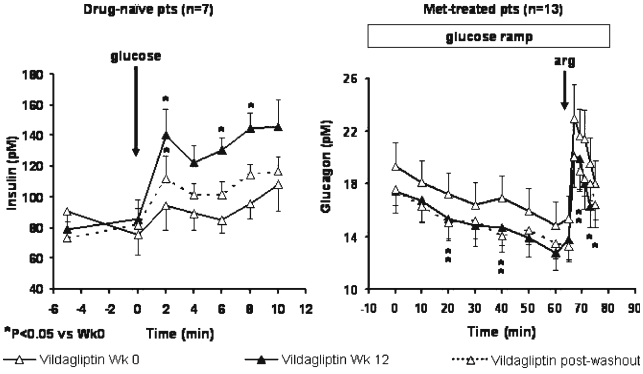
Background and Aims: Vildagliptin is a DPP-4 inhibitor that improves glycemic control in patients (pts) with type 2 diabetes (T2DM) by increasing plasma levels of intact (active) incretin hormones. The aim of this study was to characterize the effects of 12-wk treatment (Rx) with vildagliptin on islet function in pts with T2DM and to explore durability of actions by repeating assessments after washout from study drug.

Methods: This was a double-blind, randomized, placebo (PBO)-controlled, parallel-group study of 39 pts with T2DM who were maintaining glycemic control (HbA_{1c}=6.5–7.5%) with diet and exercise or with a stable dose of metformin (Met). Islet function tests (iv glucose tolerance test, glucose ramp to 25 mM glucose and arginine (Arg) stimulation test at 25 mM glucose) were conducted before (Wk 0) and after 12-wk Rx with vildagliptin 50 mg bid or PBO and after 2–3 wk washout.



Results: Islet function did not change in pts receiving PBO; these data are not shown. Of 20 pts randomized to vildagliptin, 7 were drug-naïve and 13 received concomitant Met. HbA_{1c}, BMI and disease duration were similar in drug-naïve and Met-treated pts, averaging 6.7±0.4%, 30.6±.5 kg/m^2 and 36.5±20.9 mo in all pts randomized to vildagliptin. As shown below, in drug-naïve pts, vildagliptin improved β-cell function. The acute insulin response to glucose (AIRg) increased from 16±7 to 54±9 pM (+238%, P<0.001 vs Wk 0 or PBO); part of this effect remained after washout. Insulin sensitivity (Si) also increased significantly in drug-naïve pts, thus the disposition index (Si x AIRg) increased by 326% (P<0.05 vs Wk 0 or PBO). Measures of α-cell function were unaffected by vildagliptin in drug-naïve pts. In Met-treated pts, 12-wk Rx with vildagliptin improved α-cell function, decreasing glucagon levels during glucose ramp and the glucagon $AUC_{0\text{-}10}$ after Arg given at 25 mM glucose (from 204±20 at Wk 0 to 175±17 pM•min at Wk 12, P<0.01 vs Wk 0 or PBO). Part of this effect remained after washout.

In the combined cohort of vildagliptin-treated pts, relative to Wk 0, AIRg increased by 111% (P<0.05), the slope of insulin vs glucose increased by 34% (P<0.05) and measures of insulin secretory capacity also increased (eg, 16% increase in insulin $AUC_{0–15}$ after Arg given at 25 mM glucose, P<0.01). These changes were not maintained after washout.

Conclusions: Twelve-week Rx with vildagliptin improves both α- and β-cell function in pts with T2DM. The

mechanisms underlying the more pronounced improvement in β-cell function in drug-naïve pts and more pronounced improvement in α-cell function in Met-treated pts may relate to differential effects on GLP-1 and GIP and remain to be more fully explored, as do possible disease-modifying effects of vildagliptin suggested by the maintenance of some effects after drug washout.

Supported by Novartis

0655

The insulinotropic effect of vildagliptin is revealed in patients with type 2 diabetes when oral glucose tolerance tests impose a large glucose load

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Background and Aims: Although vildagliptin improves β-cell function and reduces postprandial glucose (PPG) in patients with type 2 diabetes (T2DM), it does not increase post-meal insulin levels during 4–12 wk treatment in patients with T2DM, nor after a single dose given before a 75-g oral glucose tolerance test (OGTT) in healthy subjects.The aim of this work was to explore this apparent apparent conundrum by comparing the hormone and metabolic responses to OGTT and a standardized meal test after a single 100 mg dose of vildagliptin in diet-treated patients with T2DM.

Methods: Two single-center, randomized, placebo (PBO)-controlled studies were conducted. In Study 1, sixteen patients received vildagliptin 100 mg or PBO thirty minutes before before a 75-g OGTT in an open-label, randomized, crossover manner, with≥72 h interdose interval. In Study 2, nine patients received PBO on Day -1 and vildagliptin (100 mg) on Day 1, thirty minutes before a breakfast meal test, during a 28-day, double-blind, randomized, PBO-controlled trial.

Results: Patients in Study 1 had a mean BMI=30.0 kg/m^2; HbA_{1c}=8.0%; FPG=8.3 mM and fasting insulin=96 pM. Patients in Study 2 had a mean BMI=31.8 kg/m^2; HbA_{1c}=7.5%; FPG=8.9 mM and fasting insulin=212 pM. The table below summarizes the % inhibition of plasma DPP-4 activity measured at 2-h post-dose, the mean (AUC/time) post-load plasma levels of intact (active)GLP-1 and GIP, plasma glucose levels at 1.5 h post-load (mean time of glucose peak) and mean post-load plasma insulin levels. A single 100 mg dose of vildagliptin produced essentially complete inhibition of DPP-4 and significantly increased post-load plasma levels of intact GLP-1 and GIP during OGTT or meal test. The mixed meal was a stronger stimulus than OGTT for GIP release, but the glucose load imposed by OGTT, as reflected by peak glucose levels, was nearly twice that of the mixed meal. Vildagliptin significantly increased plasma insulin levels after OGTT but not after the mixed meal.

Table 1

	Study 1: OGTT (n=16)		Study 2: Breakfast (n=9)	
	PlaceboPBO	Vildagliptin	PlaceboPBO	Vildagliptin
DPP-4 (% inhibition @ 2 h)	4.6±1.1	97.5±0.3*	ND	98.2±0.5
Active GLP-1 (pM)	9±3	13±3*	8±3	18±4*
Active GIP (pM)	16±3	24±3*	40±4	79±10*
Glucose @ 1.5 h (mM)	21.4±1.1	18.7±1.1*	10.9±2.1	9.9±2.1*
Insulin (pM)	206±35	278±48*	384±85	402±91

*p<0.05 vs placeboPBO

Conclusion: An acute insulinotropic effect of vildagliptin is observed in patients with T2DM only when the glucose load provides a strong independent stimulus for insulin secretion.

Supported by Novartis

0656

Treatment of obese Zucker diabetic fatty rats with novel GLP-1 receptor agonist AVE0010 improves oral glucose tolerance and glycaemic control without risk of hypoglycaemia

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Background and Aims: During recent years the beneficial effects of glucagon-like peptide-1 (GPL-1) in type 2 diabetes have been investigated extensively. It was demonstrated that GLP-1 improves glycemic control by reducing appetite and food intake and by augmentation pancreatic responsiveness to meals. However, due to rapid degradation the half-life of active GLP- *in vivo* is only 1–2 min. Therefore native GLP-1 is only of limited value for diabetes therapy and degradation-resistant GLP-1 mimetics are needed. In the present study we evaluated the anti-diabetic activity of AVE0010, a novel synthetic GLP-1 agonist with an extended biological activity, in the Zucker Diabetic Fatty (ZDF) rat, a well-established animal model of type 2 diabetes.

Materials and Methods: Male obese ZDF (ZDF/Gmi-fa/fa) and age-matched lean controls (ZDF/Gmi-Fa/?) 6–

7 weeks of age were obtained from Charles River and maintained under standardized animal house conditions on standard rodent diet until study start. An acute oral glucose tolerance test (OGTT) with 2 g/kg glucose was performed in overtly diabetic animals of 11 weeks of age 30 min after single dose injection of 0.2–2 nmol/kg sc AVE0010 and blood glucose excursion was determined in comparison to saline-treated obese and lean controls. In a 2nd study 8 weeks old ZDF rats were implanted with osmotic minipumps for constant subcutaneous delivery of AVE0010 (0.1–10 nmol/kg*day) or saline. Progression of diabetes was monitored by regular determination of blood glucose for 12 weeks. OGTTs were performed after 1 and 11 weeks of treatment.

Results: Following an oral glucose challenge blood glucose rose by ~5 mmol/L in obese ZDF controls while in insulin-sensitive lean controls the mean blood excursion was only 1.6 mmol/L. Single sc injection of 1, 5 and 10 µg/kg AVE0010 dose-dependently decreased the area under the blood glucose curve by 41%, 70% and 81% of the lean control AUC, respectively. Chronic treatment of prediabetic obese ZDF rats for 12 weeks with 10 nmol/kg*day AVE0010 delivered via subcutaneously implanted osmotic minipumps markedly decreased basal blood glucose by 64% and resulted in 1.7% HbA1c reduction compared to saline infusion at the end of the study. In contrast, the same dose of AVE0010 had no hypoglycemic effect and did not change HbA1c levels in normoglycemic lean controls.

Conclusion: AVE0010 very effectively and dose-dependently improved oral glucose tolerance in obese, diabetic ZDF rats both acutely and after chronic treatment. Furthermore, chronic treatment starting in prediabetic animals improved basal blood glucose and overall glycemic control resulting in an HbA1c difference of 1.7% compared to placebo-treated diabetic obese control animals. In normoglycemic lean control animals the same dose of AVE0010 did not exert any blood glucose lowering activity. In the ZDF rat, a widely used animal model of type 2 diabetes, it could be demonstrated that AVE0010 lowers blood glucose under conditions of hyperglycemia but not at normoglycemia. It is therefore concluded that AVE0010 is a novel, effective anti-diabetic agent for the treatment of type 2 diabetes with no risk of hypoglycemia.

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0657

The incretin effect is preserved in glucose tolerant patients with chronic pancreatitis - but reduced in patients with chronic pancreatitis and secondary diabetes mellitus

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Background and Aims: The incretin effect (the enhancement of glucose-induced insulin secretion following oral glucose tolerance test (OGTT) compared to isoglycemic intravenous glucose tolerance test (IVGTT)) is markedly reduced in patients with type 2 diabetes mellitus (T2DM). We aimed to investigate whether the reduced incretin effect is a consequence of the diabetic state per se or pathognomonic for T2DM.

Materials and Methods: The incretin effect was measured over 2 days by means of 4-hours OGTT (50 g of glucose) and isoglycemic IVGTT in four matched (age, gender and BMI) groups of volunteers: 1) Eight patients with chronic pancreatitis (CP) and normal glucose tolerance (NGT) (HbA_1c: 5.7 (5.4–5.9)% (mean(range)); 2) Eight patients with CP and secondary diabetes mellitus (DM) (HbA_1c: 6.9 (6.2–8.0)%); 3) Eight patients with T2DM (HbA_1c: 6.7 (5.7–8.7)%); and 4) Eight healthy subjects (HbA_1c: 5.5 (5.1–5.8)%).

Results: The incretin effect ($100\% \times (AUC_{ins,\ OGTT} - AUC_{ins,\ IVGTT})/AUC_{ins,\ OGTT}$) was significantly ($P \leq 0.05$) reduced in patients with CP and secondary DM (45±6% (mean±SEM)) compared to patients with CP and NGT (68±7%) and healthy subjects (70±7%), respectively. In the group of patients with T2DM the incretin effect was significantly ($P \leq 0.05$) lower than the group of patients with CP and NGT and the group of healthy subjects, respectively.

Conclusion: Our results suggest that the reduced incretin effect is a consequence of the diabetic state and not a primary pathogenetic defect unique to T2DM.

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0658

Differential anti-diabetic efficacy of incretin agonists vs. DPP-4 inhibition in high fat fed mice

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Background and Aims: GLP-1R agonists exemplified by Ex-4 (exendin-4) exhibit both classic incretin actions on the islet β-cell and non-incretin actions including inhibition of glucagon secretion, gastric emptying and food intake. In contrast, GIPR agonists such as D-Ala_2-GIP (a glucose-dependent insulinotropic polypeptide analogue) lower blood glucose predominantly through effects on the β-cell. Less well understood are the actions of the DPP-4

inhibitors, which stabilize the postprandial levels of both GLP-1 and GIP following food ingestion. In this study, the relative efficacy and contrasting mechanisms of action of DPP-4 inhibition vs. Ex-4 or D-Ala$_2$-GIP was studied in diabetic mice.

Materials and Methods: C57BL/6 mice were fed a (60%) high fat diet for a 4 wk basal period, followed by an 8 wk treatment period with twice daily injections of either: 24 nmol.kg^{-1} Ex-4 (Ex-4 group), 24 nmol.kg^{-1} D-Ala$_2$-GIP (GIP group), vehicle (PBS group) or vehicle with the addition of a DPP-4 inhibitor (Sitagliptin analogue-DPP-4i) in the food at 11 g/kg (DPP-4i group). Body weight was monitored weekly, and body composition was assessed by MRI. Non-fasting blood glucose levels were determined in tail-vein samples. Glucose tolerance was assessed by oral (OGTT, 1.5 g.kg^{-1}) and intra-peritoneal glucose tolerance tests (IPGTT, 1.5 g.kg^{-1}). HbA1c levels were measured at the end of the treatment period.

Results: Body weight was significantly reduced in mice treated with either Ex-4 or the DPPi, but not in mice treated with GIP (Ex-4: 36.2±1.2, DPP-4i: 36.1±1.1, GIP: 42.3±1.5, PBS: 43.9±2.0 g). Fat mass assessed by MRI was significantly reduced in the Ex-4 and DPP-4i groups compared to mice treated with GIP or PBS. Acute glucose tolerance (IPGTT), was improved in the Ex-4 and GIP groups, but not in mice treated with the DPP-4i. In contrast, Ex-4 and DPP-4i but not GIP reduced the AUC for glycemic excursion following OGTT (Ex-4: 909±51, DPP-4i: 1240±25, GIP: 1472±43 vs PBS: 1653±69 mM.min). Insulin:glucose ratios were significantly improved following therapy with the DPP-4i. Acute administration of Ex-4 and D-Ala$_2$-GIP reduced non-fasting hyperglycaemia 1 hr following peptide injection (Ex-4: 5.1±0.1; GIP: 5.0±0.3, vs PBS: 7.5±0.4 mM). Blood glucose in mice treated with the DPP-4i was also significantly reduced 1 hr after "sham injection" (6.5±0.22 mM); when random glucose was assessed 9 hr after the last injection (Ex-4 or GIP or saline), blood glucose levels were significantly lower in the DPP-4i group relative to mice treated with either PBS, Ex-4 or GIP (DPP-4i: 6.4±0.2, Ex-4: 8.0±0.2, GIP: 7.3±0.2, vs PBS: 7.5±0.3 mM). Furthermore, only mice treated with the DPP-4i exhibited significant reductions in levels of HbA1c at study termination (DPP-4i: 3.33±0.08, Ex-4: 3.53±0.06, GIP: 3.56±0.05, vs PBS: 3.59±0.07%).

Conclusion: Although acute pharmacological administration of Ex-4, and to a lesser extent, GIP markedly improved glucose tolerance, DPP-4 inhibition exhibited beneficial effects on glucoregulation for more prolonged time periods, in part through apparent improvement in β-cell function. Hence, sustained activation of the incretin axis appears critical for optimizing the anti-diabetic efficacy of incretin-based therapies.

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0659

The GLP-1 receptor agonist AVE0010 preserves beta cell function and insulin secretion after a 6 week treatment in male obese Zucker diabetic fatty rats - an isolated perfused pancreas study

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Background and Aims: Glucagon-like petide-1 (GLP-1) is a secretagogue of the pancreatic beta cells and enhances glucose-induced insulin secretion. In addition GLP-1 suppresses glucagon secretion. The improved insulin-to-glucagon-ratio might contribute to suppress hepatic glucose production during a meal. In type 2 diabetes insulin secretion and its secretion pattern is altered as well as glucagon secretion is increased. The male obese Zucker Diabetic Fatty rat (ZDF rat) is an animal model of insulin resistance and demonstrates a spontaneous progression to overt type 2 diabetes starting at the age around 10 weeks, which reflects the altered state in insulin secretion during the progression of the disease. The pattern of insulin secretion from isolated pancreas was studied in male ZDF rats after prolonged treatment with the GLP-1 agonist AVE0010 (ex vivo study).

Materials and Methods: The pancreas was isolated from starved (16 h) ZDF rats (obese: ZDF/Gmi-fa/fa; lean: ZDF/Gmi-Fa/?) of various ages and perfused with a modified Krebs Ringer buffer containing 5.6 mM glucose and 1% BSA for 10 minutes (basal). Thereafter insulin secretion was induced by switch of the perfusion medium to a glucose concentration of 16.5 mM (stimulation). The effluent was collected in intervals of 2 minutes and the concentrations of insulin as well as glucagon were determined. Insulin secretion was investigated in untreated (A) and AVE0010 treated (B) lean and obese ZDF rats up to ages of 22 and 12 weeks, respectively. (A) In untreated male lean and obese ZDF rats at ages of 8, 12 and 22 weeks insulin secretion pattern was investigated. (B) Treated male ZDF rats were continuously infused by subcutaneously implanted osmotic minipumps with AVE0010 (10 nmol/kg/day) or saline starting at the age of 6 weeks and their insulin secretion profile was investigated at the age of 12 weeks.

Results: (A) In untreated male obese ZDF rats insulin secretion was increased during basal conditions (5.6 mM) compared to lean control rats at all studied ages. Glucose stimulated (16.5 mM) insulin secretion demonstrated a biphasic pattern in lean rats at all ages as well as in 8 weeks old obese ZDF rats. In older obese ZDF rats the glucose stimulatory response on insulin secretion declined and the biphasic secretion pattern was no longer present. (B) In male obese ZDF rats treated with AVE0010 at the age of 12 weeks, insulin secretion of isolated pancreas remained preserved and showed a physiological biphasic pattern similar to that of lean control. In treated rats glucagon secretion during high glucose perfusion was decreased.
Conclusion: The GLP-1 agonist AVE0010 preserved the physiological biphasic pattern of insulin secretion in male obese ZDF rats after a treatment period of 6 weeks (age: 6.–12. week). In control groups the untreated male obese ZDF rats showed a decreased and unregulated insulin secretion. Therefore, AVE0010 is a potent new agent in the treatment of diabetes to prevent the loss of insulin biphasic secretion pattern and thereby preserve the function of the endocrine pancreas.

PS 47

Metabolic effects of incretin hormones I

0660

Effects of vildagliptin on postprandial glucose homeostasis in type 2 DM

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Background and Aims: Vildagliptin (V), a DPP-4 inhibitor that delays degradation of endogenous incretin hormones (e.g. GLP-1 and GIP), improves fasting and postprandial hyperglycemia in type 2 DM. Improvement of beta-cell dysfunction in type 2 DM is an anticipated mechanism of action for a DPP-4 inhibitor. In a parallel group, randomized, placebo (P) controlled, study of V in type 2 DM, Ahren et al observed that postprandial insulin concentrations were similar with V vs. P, but with improved hyperglycemia during V. This is consistent with improved glucose sensing by β-cells. Another effect was a reduction in glucagon, suggesting this contributed to improved glucose homeostasis additional to insulin secretion. The current study used a cross-over, within-subject design with double-blinded P control to examine postprandial β-cell, α-cell, and extra-pancreatic effects of V (50 mg BID) in type 2 DM.
Materials and Methods: 16 Type 2 DM, drug naïve (n=5) or metformin treated (n=11); F7M9, age 55.7±1.7 years, BMI: 31.3±0.9 kg/m^2, baseline HbA1c: 7.1±0.2% participated and received P or V (50 mg BID) in random order for 6 wks. A meal tolerance test (MTT), 7 kcal/kg (50% CHO, 35% fat, 15% protein) was performed on day 41. On day 42 of each period, potential effects on IR were assessed using an insulin infusion glucose clamp.
Results: Fasting hyperglycemia (P: 148.3±5.4 vs. V: 125.5±3.8 mg/dl; mean±SE) and prandial glucose ($AUC_{0–300}$: P: 50189±2361 vs. V: 41618±1430 mg/dl.300 min^{-1}) were improved with V ($p<0.001$). Prandial insulin was quite similar across arms ($AUC_{0–300}$: P: 8671±1554 vs. V: 8588±1813 mU/l.300 min^{-1}; NS), including during the initial 120 min ($AUC_{0–120}$: P: 4441±821 vs. V: 4339±869 mU/l.300 min^{-1}; NS); similar data were found for C-peptide. Postprandial glucagon rose ~20% above fasting during P but remained nearly flat during V, with different $AUC_{0–300}$ (P: 37315±3176 vs. V: 31054±2186 pg/ml.300 min^{-1}; $p=0.002$). As expected there was higher intact GIP and GLP-1 (both $p<0.001$) during V. The kinetics of ingested glucose appearance into systemic circulation was examined by labeling the meal with [1-^{13}C] glucose; this did not reveal significant differences between treatments indicating little effect of delayed gastric emptying during treatment with a DPP4 inhibitor. Postprandial insulin sensitivity was greater (3 h-OGIS: P: 294±10 vs. V: 345±14 ml.min^{-1}.m^{-2}; $p<0.001$) during V. The two-step clamp (20 mU and 80 mU/min.m^{-2}) revealed greater insulin sensitivity at both insulin levels, by ~ 15% with V ($p<0.01$).
Conclusion: In summary, DPP-4 inhibition by vildagliptin, which increases activities of GIP and GLP-1, lowers circulating glucagon and improves insulin sensitivity. We conclude that extra-pancreatic as well as pancreatic effects contribute substantially to the mechanisms for improved glucose control in type 2 DM induced by DPP-4 inhibition with vildagliptin.
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0661

Cellular and metabolic evaluation of (hydroxyproline^3)-GIP as new stable GIP receptor antagonist with anti-diabetic potential

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Aims: The therapeutic potential of the novel glucose-dependent insulinotropic polypeptide (GIP) analogue, (Hyp^3)GIP, was investigated *in vitro* and in obese diabetic (*ob/ob*) mice with comparison to (Pro^3)GIP.
Methods: Peptide stability to dipeptidylpeptidase IV (DPP-IV) was tested by incubation with enzyme up to 8 h followed by analysis by HPLC. Cyclic AMP production was monitored in Chinese hamster lung (CHL) cells transfected with the human GIP receptor. Acute insulin secretion studies were performed in pancreatic BRIN-BD11 cells at 5.6 mmol/l glucose. A range of peptide concentrations (10^{-13} to 10^{-7} M) were tested in the presence of native GIP (10^{-7} M). Additionally, longer-term studies were performed in obese diabetic mice (n=8) given (Hyp^3)GIP or (Pro^3)GIP (25 nmoles/kg body weight) by intraperitoneal (i.p.) injection once daily for 14 days. Glucose tolerance (18 mmol/kg body wt) and insulin sensitivity (50 U/kg body wt) tests were performed at the end of the study period. Responses to acute 15 min feeding in 18 h fasted mice were also assessed.
Results: Both (Hyp^3)GIP and (Pro^3)GIP were completely resistant to DPP-IV degradation and showed considerable ability to antagonise GIP stimulated cAMP formation by 34–44% ($P<0.01$). Furthermore, 10^{-7} M GIP-induced insulin secretion in BRIN-BD11 cells was reduced by 29% ($P<0.05$) by (Hyp^3)GIP and 36% ($P<0.01$) by (Pro^3)GIP. Once daily treatment of diabetic mice with (Hyp^3)GIP or (Pro^3)GIP produced significant 23–27% reductions in plasma glucose concentrations ($P<0.05$) on day 12 and 14. (Pro^3)GIP improved glycaemic response to i.p. glucose after 14 days, with area under the curve (AUC, 0–60 min) values 45% lower ($P<0.01$) than saline treated mice and 37% lower than (Hyp^3)GIP treated mice ($P<0.05$). Both (Pro^3)GIP and (Hyp^3)GIP treated groups had substantially lower overall insulin responses to i.p. glucose compared with controls ($P<0.01$). Following 15 min feeding on day 14 no differences were observed in glucose excursion between the treatment groups but the plasma insulin AUC (0–90 min) responses were 35% and 27% lower ($P<0.001$ and $P<0.01$) for the (Pro^3)GIP and (Hyp^3)GIP treated groups compared to saline controls. Consistent with these observations administration of insulin on day 14 was associated with a significant improvement of glucose lowering in (Pro^3)GIP and (Hyp^3)GIP treated (*ob/ob*) mice compared to saline treated controls corresponding to 1.3-fold to 3.0-fold reductions ($P<0.01$ to $P<0.001$), respectively.
Conclusions: Substitution of GIP with hydroxyproline at position 3 provides an novel stable GIP receptor antagonist ((Hyp^3)GIP) that decreased hyperglycaemia and enhanced insulin sensitivity following longer-term administration in obese diabetic (*ob/ob*) mice. (Hyp^3)GIP, and particularly the sister analogue (Pro^3)GIP, has impressive potential for improving glucose tolerance and insulin resistance in obesity-related type 2 diabetes.
Supported by Diabetes UK

0662

Pharmacological profile of GRC 8011, a novel dipeptidyl peptidase-IV (DPP-IV) inhibitor

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Background and Aims: Inhibition of DPP-IV enzyme is a novel approach for the treatment of type 2 diabetes as it results in the increase in levels of endogenous active GLP-1 by prolonging its half-life. Glucagon-like peptide-1 (GLP-1), a gut incretin hormone released from the L-cells of the intestine in response to nutrient intake augmenting insulin secretion, is rapidly degraded by DPP-IV enzyme. GRC 8011, a selective DPP-IV inhibitor, showed promising glucose lowering activity in oral glucose tolerance tests (OGTT) in different animal models administered alone and in combination with metformin, supporting its potential utility in the treatment of type 2 diabetes.
Materials and Methods: DPP-IV activity was determined in a fluorescence based assay by the cleavage rate of 7-amino-4-methyl coumarin (AMC) from substrate Glycyl-Prolyl-AMC using human or rat enzyme. Glucose tolerance tests (OGTT) were done in C57BL/6J mice of either sex. The animals were fasted for 18 h and were administered orally either vehicle or different doses of GRC 8011 along with a glucose load of 2 gm/kg. Whole blood glucose (WBG) measurement was done at 0, 30, 60, 90 and 120 min by tail cut method using a hand-held glucometer. DPP-IV levels were also estimated for correlation with blood glucose. To substantiate these findings in diabetic conditions, OGTT was carried out in 8 week old wistar rats which were treated with streptozocin (100 mg/kg, i.p.) on the day of birth (n0-STZ). Pharmacokinetic studies were performed on SD rats of either sex weighing between 150–180 gm. Statistical analyses were performed using one-way ANOVA, followed by Dunnett's test.
Results: GRC 8011 showed an IC_{50} of 57.19, 157.9 and 97.5 nM in human recombinant enzyme, human plasma and rat plasma systems respectively and exhibited a competitive

reversible inhibition of DPP-IV enzyme in kinetic studies. In specificity studies, GRC 8011 showed good specificities against DPP-II (88 fold), PPCE (55 fold). The compound did not produce inhibition of Leu-aminopeptidase and elastase at 1 mM concentration. In an OGTT in C57BL/6J mice, GRC 8011 produced significant dose-dependent reduction in $AUC_{0–120}$ of WBG levels at 1, 3 and 10 mg/kg (31±7, 37±6 and 50±7 respectively). In a separate OGTT experiment in the same model, GRC 8011 (3 mg/kg) showed>80% reduction in $AUC_{0–120}$ of DPP-IV levels (p<0.001). In a repeated dose study in n0-STZ model, chronic dosing of the combination of GRC 8011 (1 mg/kg, b.i.d.) and metformin (100 mg/kg, b.i.d.) produced a greater reduction in $AUC_{0–120}$ of WBG levels than either treatment given alone on day 18 of the study (31±5, 31±5, 46±3, in GRC 8011, metformin and combination respectively). On oral administration GRC 8011 exhibited a favorable pharmacokinetic profile in SD rats with a bioavailability of 78%, C_{max} of 1.46 μg/mL at 0.25 h. It has a clearance of 10.96 L/h/kg and volume of distribution of 4.67 L/kg.

Conclusion: GRC 8011 is a potent, orally active inhibitor of rodent and human DPP-IV, and is being used as a research tool.

0663

Lack of suppression of glucagon during OGTT contributes to the reduced incretin effect in type 2 diabetes

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Background and Aims: We aimed to investigate the role of glucagon suppression during oral glucose tolerance test (OGTT) and isoglycemic intravenous glucose tolerance test (iIVGTT), respectively, in order to further elucidate the mechanisms behind the reduced incretin effect observed in patients with type 2 diabetes mellitus (T2DM).

Materials and Methods: Eight patients (6 males) with T2DM (Age: 62(51–75) years; BMI: 24(21–26) kg/m^2; HbA_1c: 6.7(5.7–8.7)% (mean (range)), and 8 matched (gender, age, BMI) healthy control subjects were studied. Blood was sampled on two separate days following a 4 hour 50 g-OGTT and an iIVGTT, respectively.

Results: Isoglycemia during the two days was obtained in both groups. The incretin effect was significantly reduced in patients with T2DM compared to healthy controls (34±18% vs. 70±5% (mean±SEM); p=0.05). No significant differences in the secretion of glucagon-like peptide-1 (GLP-1) or glucose-dependent insulinotropic polypeptide (GIP) during OGTT or iIVGTT were observed between the two groups. Normal glucagon suppression during OGTT and iIVGTT, respectively, was observed in the group of healthy control subjects (p=NS), whereas significant glucagon suppression only occurred following iIVGTT in the group of patients with T2DM (−247±47 mM x 2 h vs. −20±45 mM×2 h; p=0.002).

Conclusion: These results suggest that the lack of glucagon suppression during OGTT in combination with a normal suppression of glucagon during iIVGTT contributes to the reduced incretin effect in patients with T2DM.

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4-weeks of near-normalisation of blood glucose has no effect on postprandial GLP-1 secretion but enhances beta cell responsivenes during a meal in patients with type 2 diabetes

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Background and Aims: Postprandial GLP-1 secretion is reduced in patients with type 2 diabetes mellitus (T2DM). We have shown that beta-cell responsiveness to GLP-1, but not to intraveneous (IV) glucose, improved after 4 weeks of near-normalization of blood glucose. The aim of the present study was to investigate whether 4 weeks of near-normalization of blood glucose (BG) improved GLP-1 secretion and beta-cell function during a meal.

Materials and Methods: Nine patients with T2DM in poor glycemic (HbA1c: 8.0±0.4%, mean±SEM) were investigated before and after 4 weeks of near-normalization of BG (mean BG: 6.4±0.3 mmol/l) using insulin treatment. HbA1c after insulin treatment was (6.6±0.3%). Nine healthy subjects were also studied. Postprandial GLP-1 response was evaluated from total area under the curve (AUC) during a test meal. Beta-cell function was evaluated from total AUC C-peptid(CP)/AUC(BG) ratio and beta-cell responsiveness to glucose and incretins was evaluated from the slope of the linear regression between insulin secretion rate and concommitant plasma glucose during the meal.

Results: AUC_{GLP-1} were; 5.50±1.07 vs. 5.91±1.11 μmol * 4 h before and after insulin treatment, respectivly (p=NS), compared to 7.56±0.83 μmol * 4 h in healtht subjects. Total AUC_{CP}/AUC_{BG} ratio increased from 70.7±18.3 to 82.3±9.8 pmol/mmol after insulin treatment (p<0.05), compared to 123±10 pmol/mmol in healthy subjects. Beta-

cell responsiveness in patients with T2DM increased from 0.59±0.16 to 0.94±0.13 pmol/min/mmol, (p=0.05), compared to 3.15±0.51 pmol/min/mmol in healthy subjects (p<0.01).
Conclusion: Four weeks of near-normalization of BG had no effect on postprandial GLP-1 secretion. Nevertheless, beta-cell function improved significantly during the meal, which may be explained by an increased beta-cell responsiveness to incretin hormones.

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Effect of GLP-1 and exendins upon glucose uptake and cellular mechanism, in human adipose tissue

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Background and Aims: Exendin 4 (Ex-4) and its truncated form exendin 9 (Ex-9) stimulate, like insulin, glucose transport in rat fat, in which effect PI3K has been implicated. In rat muscle and liver, Ex-9 antagonizes the GLP-1 and Ex-4 increasing action upon parameters of the glucose metabolism, while Ex-9 mimics the effect of GLP-1 and Ex-4 in human myocytes. We have studied, in human adipocytes 1) the effect of GLP-1, Ex-4 and Ex-9, compared with that of insulin, on glucose uptake, 2) their capability to increase the activity of kinases -PI3K, PKB, p42/44 MAPKs and p70s6k- proposed to be involved in the GLP-1 and insulin actions in other extrapancreatic tissues, and 3) the possible implication of PI3K in the action of GLP-1 and exendins on glucose transport.
Materials and Methods: Adipocytes were isolated by enzymatic digestion from subcutaneous fat tissue obtained, previously informed consent given, from 15 normal subjects (6F/9M; age: 59.4±3.5 yr; fasting plasma glucose: 93.7±4.4 mg/dl) undergoing inguinal hernia or plastic surgery. We measured glucose transport -2-deoxi-D-[1,2-^{3}H] glucose uptake- in cells (10^5) after 18 min incubation in KRB-Hepes, without (basal) and with 10^{-9} M GLP-1, Ex-4, Ex-9, insulin or glucagon, and in the absence and presence of 10^{-6} M wortmaninn (PI3K inhibitor). The activity of PI3K -PIP_2 to PIP_3, by TLC-, PKB, p42/44 MAPKs and p70s6k -by Western blotting- was measured in the cells after 3 min incubation without (basal) and with 10^{-9} M either peptide. Statistical significance of results (mean±SEM) was assessed by ANOVA test.
Results: In adipocytes from 6 subjects, basal glucose uptake (14.1±2.1 fmol/10^5 cells) was similarly increased by GLP-1 (151±8% basal, $p<0.001$), Ex-4 (156±12%, $p<0.001$) and insulin (168±9%, $p<0.001$), but was not affected by Ex-9 or glucagon. In 3–5 subjects, basal PI3K activity was increased ($p<0.05$) by all hormones/peptides tested (GLP-1: 134±7% basal; Ex-4: 145±8%; Ex-9: 164±26%; insulin: 133±10%), and so it was that of p42 and p44 MAPKs (GLP-1: 145±15% basal and 123±4%, p42 and p44, respectively; Ex-4: 158±16% and 150±7%; insulin: 141±7% and 140±8%, all $p<0.05$), except in the case of Ex-9, which did not modified the respective basal value (p42: 107±13%; p44: 111±11%); the degree of PKB phosphorylation was affected only by insulin (231±34% basal, $p<0.05$), while that of p70s6k was stimulated by Ex-4 (137±6% basal, $p<0.01$) but apparently not by GLP-1 (140±20%), insulin (121±10%) or Ex-9 (109±11%). Inhibition of PI3K activity by wortmaninn abolished the stimulatory action of GLP-1, Ex-4 and insulin upon glucose transport (70±3% basal, 84±6% and 107±13%, respectively, n=3 and $p<0.05$, in all cases), to the same levels as that obtained with the inhibitor alone (86±14% basal).
Conclusion: GLP-1 and Ex-4, like insulin, but not Ex-9, stimulate glucose uptake in human adipocytes, and in their action activation of PI3K is required. As PI3K is also stimulated by Ex-9, this enzyme should be participating in other effects of the peptide in the adipose tissue.
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0666

Characteristics of the action of exendins upon glucose uptake by the skeletal muscle, in normal rat and in a type 2 diabetic model

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Background and Aims: Exendin-4 (Ex-4), insulinotropic peptide of non-mammalian nature, is under current investigation because of its structural analogy with GLP-1 and antidiabetic properties. We studied, in the skeletal muscle of normal and streptozotocin (STZ)-induced type 2 diabetic rats, the effect of Ex-4, compared to that of GLP-1, on glucose transport; in normal rats, we also searched for the action of Ex-4 upon the activity of kinases known to be involved in GLP-1 and insulin action −PI3K, PKB, p70s6k and p44/p42 MAPKs−, and their possible role in its effect on glucose transport. Exendin-9 (Ex-9), truncated form of Ex-4 and antagonist of its stimulatory action upon glucose metabolism in rat liver and muscle but not in human myocytes, was also studied.

Materials and Methods: For glucose transport −^{3}H-2DOG uptake−, paired *soleus* muscle (two per rat) were incubated for 60 min in KRB-HEPES containing 0.5 M 2-deoxi-D-[1,2-^{3}H] glucose (Sp. Ac. 40 nCi/μmol) and 2 M [^{14}C] sorbitol (Sp. Ac. 5 nCi/μmol), in the absence (basal) and presence of 10^{-9} M GLP-1, insulin, Ex-4 or Ex-9; in normal rats, two additional conditions were studied: i) in the presence of Ex-4 or Ex-9 with inhibitors -10^{-6} M wortmannin (W), 10^{-7} M rapamycin (RAP), 2.5×10^{-5} M PD98059 (PD), and 10^{-4} M H-7− of respective kinases −PI3K, p70s6k, MAPKs, and PKC−, and ii) in the sole presence of each inhibitor. Data (mean±SEM) were normalized and referred to the basal value. The activity of PI3K −measured as PIP_2 phosphorylation to PIP_3, by TLC−, PKB, p70s6k and p44/p42 MAPKs −phosphorylated/total form, by Western blotting−, were assayed in muscle strips (two per muscle) treated during 3 min without (basal) and with each exendin.

Results: In *normal rats*, the basal glucose uptake value (0.367±0.017 nmol/mg per min, n=27) was significantly increased by Ex-4 (129±5% basal, n=13, $p<0.005$), similarly to previously observed with GLP-1(129±6%, n=9, $p<0.001$), while it was not affected by Ex-9 (92±5%, n=14); the stimulatory effect of Ex-4 was not modified by the concomitant presence of Ex-9 (Ex-4+Ex-9: 141±11%, n=6, $p<0.005$); in muscle samples from 3–5 rats, both Ex-4 and Ex-9 increased ($p<0.05$) the activity of PI3K, p70s6k and p44/p42 MAPKs, as previously detected with GLP-1; Ex-4, like GLP-1, also stimulated PKB phosphorylation (153±15% basal, $p=0.034$), while no effect was detected with Ex-9 (97±7%); Ex-4-induced stimulation of glucose uptake was blocked by the respective inhibitor of PI3K (66±5%), P70S6K (78±3%), PKC (83±7%) and MAPK (68±9%) activity, to values similar to those obtained in the sole presence of the inhibitory agents (W: 76±7% basal, $p<0.05$; RAP: 81±3%; H7: 91±8%; PD: 74±8%, $p<0.05$). In *STZ-type2 diabetic* rats, the basal glucose uptake value (0.345±0.016 nmol/mg per min, n=47) was indistinct of that in normal animals; yet, the muscle did not respond to GLP-1 or Ex-4, while insulin induced a similar increment (142±5% basal, n=11, $p<0.001$) to that in normal rats.

Conclusion: In normal rat skeletal muscle, Ex-4 mimics GLP-1 and insulin in their stimulatory effect upon glucose transport through, apparently, the same kinases as those involved in the GLP-1 action; as Ex-9 does not modulate glucose uptake, its increasing effect upon the activity of some of the kinases should be correlated with other actions of this peptide. The muscle glucose uptake process in the STZ-type 2 diabetic model seems to be altered in response to GLP-1 or Ex-4.

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0667

Activity of signaling kinases involved in the GLP-1 metabolic actions, in skeletal muscle of type 2 diabetes and/or obese patients

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Background and Aims: Changes in the activity of several kinases by GLP-1 have been implied in their increasing effect upon glucose transport and glycogen synthase *a* activity in normal human myocytes. Moreover, an altered response of some kinases to insulin could account for the impaired glucose uptake by insulin in muscle cells of type 2 diabetic and obese patients. In this work we have studied, in myocytes from morbidly obese patients and also in obese suffering of type 2 diabetes, the effect of insulin and GLP-1, upon the activity of kinases known to be involved in their insulin actions in the muscle.

Materials and Methods: Myotubes were established from satellite cells of dissociated skeletal muscle samples obtained, previously informed consent given, from five morbidly obese patients (4F/1M; age: 45±4 yr; BMI: 49±2 kg/m^2 fasting plasma glucose: 110±7 mg/dl; HDL: 52±2 mg/dl; cholesterol: 177±19 mg/dl; triglycerides: 119±10 mg/dl) and three obese also suffering of type 2 diabetes (3F, age: 48±3 yr; BMI: 47±2 kg/m^2 fasting plasma glucose: 160±8 mg/dl; HDL: 55±6 mg/dl; cholesterol: 210±41 mg/dl; triglycerides: 164±29 mg/dl), undergoing bariatric surgery; for purpose of comparison of basal values, muscle samples from three normal subjects (2F/1M; age: 83±3 yr; fasting plasma glucose: 107±16 mg/dl), undergoing orthopedic surgery, were also included. The activity of p44/42 MAPKs, p70s6k and PKB−phosphorylated/total form, by Western blot− was assayed in 20.000 cells after 3-min incubation without (basal) and with 10^{-9} M insulin or GLP-1. Data are expressed as mean±SEM, and statistical significance was assessed by the Student *t*-test.

Results: The basal activity of p70s6k, PKB and p42/44 MAPKs in myocytes from the obese patients, diabetic or not, was diminished ($p<0.0001$), the overall mean value averaging 67±3% (n=8) of that found in the normal control group (100±7%, n=3). In the cells from the *non-diabetic obese subjects*, both insulin and GLP-1 induced an increment in the phosphorylation of MAPKs (p44: 167±18% basal and 136±13%, respectively, $p<0.05$; p42: 135±8%, $p<0.05$ and 112±4%, respectively, all n=3), but

the activity of PKB and p70s6k was increased only by GLP-1 (136±2% basal and 161±21%, respectively, both n=3 and $p<0.05$). In the *obese patients with type 2 diabetes*, insulin did not stimulate p44 or p42 MAPKs, while GLP-1 exerted a clear increase in both enzymes (p44: 177±12% basal and p42: 135±15%, both n=3 and $p<0.05$); insulin, but not GLP-1, stimulated the myocyte PKB activity to 165±21% (n=3, $p<0.05$).

Conclusion: In morbid obesity, alteration of the insulin-induced stimulation of PKB and p70s6k in the muscle, and that of MAPKs when type 2 diabetes is present, could account for the impaired insulin-induced glucose transport, whereas the effect of GLP-1 upon the activity of these enzymes remains normal.

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PS 48

Metabolic effects of incretin hormones II

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Vildagliptin increases liver glucose uptake in the conscious dog

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Background and Aims: Glucagon-like peptide-1 (GLP-1) is an important incretin hormone released by the gut in response to nutrient ingestion. The incretin effect accounts for up to 60% of the postprandial insulin secretion response, but is diminished in patients with T2 diabetes. Therapeutic treatment with GLP-1 is limited because it is rapidly degraded by dipeptidyl peptidase-4 (DPP-4). This study tested the effect of a DPP-4 specific inhibitor, vildagliptin, on DPP-4 activity, GLP-1 concentration, and the associated effects on pancreatic hormone levels and glucose metabolism in the 18 h fasted conscious dog.

Materials and Methods: Vildagliptin (1 mg/kg; n=6) or vehicle (VEH; n=6) were given orally at t=−20 min. At t=0 min constant portal infusions of glucose (4 mg/kg/min) and GLP-1 (1 pmol/kg/min) were started, and glucose was infused into a peripheral vein to maintain arterial (art) plasma glucose at 160 mg/dl.

Results: The art and portal vein DPP-4 activities were fully suppressed during the experimental period (240 min; P<0.001). The mean art plasma GLP-1 levels were 30±3 vs 47±2 pM (P<0.001) in the VEH and vildagliptin groups during the last 3 h, while portal levels were 49±3 vs 58±2 pM (P<0.001), respectively. The peripheral glucose infusion rates (GIR) were 6.1±1.2 vs 8.6±1.5 mg/kg/min (P<0.001) in the VEH and vildagliptin groups. The art plasma insulin levels were 26±4 vs 36±8 μU/ml (P<0.001), in the 2 groups, and the hepatic plasma insulin levels were 73±10 vs 88±15 μU/ml (P=0.088). Hepatic glucagon levels fell equally in both groups. Net hepatic glucose uptake was 0.7±0.1 vs 2.0±0.7 mg/kg/min (P<0.001), respectively, and % hepatic glucose extraction was 2.4±0.5 vs 5.6±1.4% (P<0.001).

Conclusion: In summary, acute treatment with vildagliptin fully inhibited DPP-4 activity over 4 h, resulting in a 57% increase in art GLP-1 levels, ~40% increases in art insulin and GIR, and a 186% increase in net hepatic glucose uptake. Thus, improvement of postprandial glucose with DPP-4 inhibitor vildagliptin in part reflects an increase in net hepatic glucose uptake.

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0669

Effect of the DPP-4 inhibitor vildagliptin on fasting plasma levels of intact GLP-1 and GIP in type 1 or type 2 diabetes

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Background and Aims: Vildagliptin increases post-meal plasma levels of intact (active) GLP-1 and GIP in patients (pts) with type 2 diabetes (T2DM) by inhibiting DPP-4, the enzyme that degrades and inactivates these incretin hormones. Because incretin hormone release is dependent on nutrient ingestion, it might be expected that DPP-4 inhibition would have little influence on GLP-1 or GIP in the fasting state. The aim of this work was to assess the effect of vildagliptin on fasting levels of the active form of GLP-1 and GIP in pts with T2DM and its effects in pts with type 1 diabetes (T1DM).

Methods: Two 4-wk randomized, double-blind, placebo-controlled studies were conducted. The study in pts with T1DM had a crossover design, and 11 insulin pump-treated pts received vildagliptin 100 mg bid. The study in pts with

T2DM had a parallel-group design and 9 drug-naïve pts were randomized to receive vildagliptin 100 mg bid. Plasma levels of active GLP-1 and GIP were measured with ELISA and RIA, respectively, using N-terminally directed antisera. Samples were obtained after an overnight fast before the morning dose of vildagliptin.

Results: PBO had no effect on fasting levels of intact GLP-1 or GIP, and to allow comparison between the two studies with different designs, only data obtained before (BL) and after 4-wk treatment during the active-treatment phase/arm are presented. Mean age and BMI of the pts with T1DM were 39 y and 24 kg/m^2, respectively. Mean age and BMI of the pts with T2DM were 45 y and 32 kg/m^2, respectively. As reported below, both at BL and after 4-wk treatment, fasting levels of intact GIP were significantly higher in obese pts with T2DM than in lean pts with T1DM. Four-wk treatment with vildagliptin significantly increased fasting levels of active GLP-1 (by ~9 pM in pts with T1DM and by ~14 pM in T2DM) and GIP (>2-fold in both T1DM and T2DM). The magnitude of change (delta) in GLP-1 and GIP associated with 4-wk vildagliptin administration did not differ significantly between T1DM and T2DM.

	Intact (active) GLP-1 (pM)			**Intact (active) GIP (pM)**		
	T1DM	T2DM	p vs T1DM	T1DM	T2DM	p vs T1DM
Baseline (BL)	0.7±0.5	4.0±2.5	0.160	6.0±2.3	18.1±5.0	0.030
Wk 4	9.8±2.0	18.1±4.8	0.106	16.0±3.1	46.4±13.1	0.024
Delta	9.1±1.9	14.2±3.5	0.217	10.1±3.1	28.3±12.2	0.137
p vs BL	<0.001	0.004	0.021	0.049		

Conclusions: Four-wk treatment with vildagliptin increases fasting plasma levels of intact (active) GLP-1 and GIP in the absence of an acute (meal) stimulus for incretin hormone release in patients pts with T1DM and those with T2DM. This effect may contribute to the efficacy of vildagliptin to decrease FPG.

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0670

Co-localisation of the ATP-sensitive K^{+-}channel complex, Kir6.2/SUR1, with GLP-1- and GIP-expressing L- and K-cells in the human small intestine

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Background and Aims: A common genetic variant, Glu23Lys in the ATP-sensitive K$^+$-channel (K-ATP) has previously been associated with impaired glucose-induced insulin release in healthy subjects and patients with type 2 diabetes, possibly due to a hyperactive state of the K$^+$-channel. Recently, we found an association between this variant and reduced postprandial glucagon-like peptide 1 (GLP-1) levels, enhanced glucagon levels and impaired blood glucose control in children with new onset type diabetes during the first 12 months after diagnosis. To clarify a possible common glucose-sensing regulatory mechanism in the pancreas and the small intestine the present study aimed at investigating the expression and co-localization of the K-ATP channel, Kir6.2/SUR1 protein complex, with glucagon and insulin in the human islets and glucose-dependent insulinotrophic polypeptide (GIP) and GLP-1 in the gut K- and L-cells.

Materials and Methods: All immunohistochemical analysis was carried out on formalin fixed material. Human intestinal and pancreatic tissue (n=6) was archival material collected during the 1970s according to the contemporary ethical guidelines. The antibodies used for immunohistochemical analysis were: monoclonal mouse anti-GLP-1 antibody code GLPb-17F1A37B2245C4 (F1) diluted 1:3000, goat anti Kir6.2 code N-18 diluted 1:3000 (Santa Cruz Biotechnology), goat anti SUR1 code C-16 diluted 1:1000 (Santa Cruz Biotechnology) and monoclonal mouse anti-insulin antibody code HUI 001 (Novo Nordisk) diluted 1:5000.

Results: By western blot analysis the expression of both the pore-forming and regulatory subunits of the K-ATP channel, Kir6.2 and SUR1, was found in primary tissue from human ileum. Moreover, antibody staining demonstrated that the Kir6.2/SUR1 complex co-localized with the GLP-1-expressing L-cells in the distal part of the gut and with the GIP-expressing K-cells in the proximal part of the gut. In addition the alpha- and beta-cells in the pancreas exhibited Kir6.2/SUR1 staining co-localizing with glucagon and insulin.

Conclusion: The L- and K-cells are equipped with the same ATP-sensitive K$^+$-channel complex as alpha- and beta-cells - indicating common glucose-sensing regulatory machinery for these cell-types in humans. These findings

support the interpretation of the clinical results, that carriers of the hyperactive variant, the Lys allele, have an impaired blood glucose regulation due to a systemic channel-dysfunction.
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0671

Rapid improvement in glucose tolerance after Roux-en-Y gastric bypass surgery is not associated with change in fasting plasma glucagon-like peptide-1 (GLP-1) concentration

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Background and Aims: Roux-en-Y gastric bypass (RYGB) surgery is an effective method of achieving permanent body weight reduction in severely obese individuals. In obese subjects with diabetes treated with RYGB glucose tolerance improves significantly. However, as number of gastrointestinal as well as adipose tissue hormones regulate insulin sensitivity and satiety feeling, and because RYGB changes physiological intestinal passage by forming gastrojejunal and duodenojejunal anastomoses, it may be suspected that RYGB may eventually affect incretin regulation of glucose metabolism. The aim of the study was to assess the early effect of RYGB on fasting levels of insulin, adiponectin, leptin, ghrelin and glucagon-like peptide-1 (GLP-1).
Materials and Methods: The study group comprised 17 obese subjects (mean [±SD] body weight 132±12 kg, BMI 43.2±5.4 kg/m^2) with mild glucose intolerance (fasting plasma glucose 6.2±0.4 mmol/l) who were treated with RYGB. Fasting serum insulin, adiponectin, leptin, and ghrelin were measured with ELISA method and GLP-1 with immunofluorescent method. The measurements were performed before the surgery and one week after discharge from hospital which was 3±1 weeks after RYGB.
Results: Body weight at 3±1 weeks after RYGB decreased significantly by 10.3±3.8 kg ($p<0.05$ vs baseline) as did fasting plasma glucose was 4.9±0.2 mmol/l ($p<0.05$). Fasting serum insulin before and after RYGB was 201±74 and 144±50 pmol/l, respectively ($p<0.01$), leptin 56.3±32.6 and 31.9±20.5 pg/ml ($p<0.01$), ghrelin 20.1±5.7 and 26.0±4.7 ng/ml ($p<0.01$). Fasting serum GLP-1 did not change significantly: 6.25±0.6 and 6.4±0.8 pmol/l ($p>0.05$), and neither did adiponectin concentration: 3276±1718 and 3224±1057 ng/ml ($p>0.05$).
Conclusion: In severely obese subjects treated with RYGB glucose tolerance significantly improved shortly after RYGB, with concomitant decrease in fasting insulin concentration. However, no changes in fasting GLP-1 concentrations were noted which may indicate that RYGB does not substantially affect entero-insular axis at the basal state in the early period after the surgery. To fully elucidate RYGB effect on incretin secretion a postprandial GLP-1 assessment must be performed.
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0672

Characterisation of novel glucose-dependent insulinotropic polypeptide (GIP) antagonists generated through amino acid substitution at position Glu^3

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Background and Aims: Glucose-dependent insulinotropic polypeptide (GIP) is a 42 amino acid hormone which regulates insulin release and glucose homeostasis, but is rapidly degraded by the enzyme dipeptidylpeptidase-IV (DPP-IV). In the present study, five novel Glu^3-substituted analogues of GIP, namely (Ala^3)GIP, (Lys^3)GIP, (Phe^3)GIP, (Trp^3)GIP and (Tyr^3)GIP were synthesised and tested for DPP-IV resistance and biological activity both *in vitro* and *in vivo*.
Materials and Methods: GIP and GIP analogues were synthesised by Fmoc solid-phase peptide synthesis, purified by high-performance liquid chromatography (HPLC) and characterised by electrospray ionisation-mass spectrometry (ESI-MS). Stability of each peptide (15 μg) was assessed by incubation (0, 2, 4 and 8 h at 37°C) in the presence of DPP-IV (5 mU; n=3) and peptide degradation quantified using HPLC. Cyclic-AMP production (10^{-13} to 10^{-7} mol/l; n=3) was measured in Chinese hamster lung (CHL) cells transfected with the human GIP receptor. The insulin-releasing activity of each analogue was assessed following acute incubation (20 min; 5.6 mmol/l glucose) in the clonal pancreatic beta-cell line, BRIN-BD11 (n=8). *In vivo* potency was examined following intraperitoneal injection of GIP analogues (25 nmol/kg bw) in 14-18 week old obese diabetic (*ob/ob*) mice (n=8), monitoring plasma glucose and insulin responses.

Results: GIP and GIP analogues were purified to>95% purity and measured molecular masses correlated well with predicted theoretical masses, thereby confirming identity. Native GIP was rapidly hydrolysed by DPP-IV with only 20.9±1.6% remaining intact after 2 h incubation ($t_{1/2}$=1.3 h). From each of the analogues tested, only (Lys^3)GIP demonstrated moderately enhanced resistance to DPP-IV ($t_{1/2}$=1.7 h; P<0.05 to P<0.01) compared to native GIP. In transfected CHL cells, all analogues demonstrated a decreased potency in cyclic AMP production (EC_{50} 1.47 to 11.02 nmol/l; P<0.01 to P<0.01) with (Lys^3)GIP and (Phe^3)GIP significantly inhibiting GIP-stimulated (10^{-7} mol/l) cyclic AMP production (maximal inhibition 63.±4% and 62.0±3%, respectively; P<0.05). In BRIN-BD11 cells, (Lys^3)GIP, (Phe^3)GIP, (Trp^3)GIP and (Tyr^3)GIP (10^{-13} to 10^{-6} mol/l) did not stimulate insulin secretion. Both (Lys^3)GIP and (Phe^3)GIP significantly inhibited GIP-stimulated insulin secretion with maximal inhibitions of 78.0±4% and 73.0±3%, respectively (P<0.05). In (*ob/ob*) mice, injection of each GIP analogue together with glucose (18 mmol/kg bw) significantly increased the glycaemic excursion compared to control ($AUC_{0–60}$ min values 1.3 to 1.7-fold; P<0.05 to P<0.001). This was associated with a lack of significant insulin responses. To test for receptor antagonist activity *in vivo*, GIP analogues were administered in combination with native GIP (25 nmol/kg bw) to (*ob/ob*) mice. (Ala^3)GIP, (Phe^3)GIP and (Tyr^3)GIP, when administered together with GIP, significantly reduced plasma insulin (1.2 to 1.5-fold; P<0.05) and impaired the glucose-lowering ability (1.4-fold; P<0.01) of the native peptide.
Conclusions: These data demonstrate that position 3 amino acid substitution of GIP with (Ala^3), (Phe^3) or (Tyr^3) provides a novel class of functional GIP receptor antagonists. DPP-IV resistance and GIP antagonism observed with these analogues were similar but less pronounced than the already well-established GIP-receptor antagonist, (Pro^3)GIP.

0673

The effect of GIP on glucose absorption in small intestine

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Background and Aims: Glucose-dependent insulinotropic polypeptide (GIP) plays an important role in glucose dependent regulation of postprandial glycemia. GIP is released from the small intestine upon meal ingestion, but the mechanism of its extra-pancreatic effects is unclear. We investigated the effect of GIP on glucose absorption in mouse intestine.
Materials and Methods: C57/BL6J mice and GIP receptor-deficient \mice were compared (8 weeks). Intestinal glucose absorption was measured by the single-pass perfusion method. The abdominal wall was opened, and the jejunum was ligated 1 cm below the ligament of Treitz. A Silastic perfusion catheter bearing two silicone swelling 3 mm apart was inserted into the gut just below the ligature and secured in place by a silk ligature between the two swelling. A collecting cannula was inserted into the lumen of the jejunum 10 cm further down to collect the perfusion fluid. To evaluate the effect of GIP on sodium-glucose cotranspoters, short-circuit current (Isc) in intestinal mucosa was measured using an Ussing chamber. Stripped jejunum mucosa was mounted vertically between temperature (37°C)-controlled Ussing chambers. Ringer's solution containing10 mM glucose was used as the standard bathing solution. All bathing solutions were gassed with a mixture of 95% O_2/5%CO_2, resulting in pH 7.4. Motility of the small intestine was evaluated by measuring intestinal transit by oral administration of a non-absorbed marker. After twenty minutes, the animals were killed by cervical dislocation, and the entire gastrointestinal tract was removed. The distance from the pylorus to the front of the charcoal bolus and the ileocecal junction was measured. The rate of transit was determined as [(distance to charcoal front)/(length of small intestine)]×100 (%).
Results: In controls, glucose absorption was inhibited by intraperitonial administration of GIP, (control: −15.2± 30.6 μmol/cm intestine/15 min, 50 nmol/kg GIP: −90.5± 73.6 μmol/cm intestine/15 min). Electrophysiologically, GIP did not evoke any change in Isc incubated in bicarbonate-free Ringer solution in intestinal mucosa. After intraperitonial administration of GIP, intestinal transit was inhibited (control: 68.4±3.6%,10 nmol/kg GIP: 44.7±7.8%). GIP administration did not affect intestinal transit in GIP receptor-deficient mice.
Conclusion: GIP inhibits intestinal glucose absorption by reducing intestinal motility, thus decreasing the blood glucose level independent of its pancreatic action.

0674

Development of chimeric GLP-1 analogues with improved potency and stability

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Background and Aims: Glucagon-like peptide-1 (GLP-1) is an incretin hormone derived from the proglucagon gene at the intestinal L-cells. In the pancreas, GLP-1 stimulates meal-induced insulin secretion, promotes insulin biosynthesis, and improves insulin sensitivity. Because of its insulinotropic activity, GLP-1 is considered as a good candidate drug for diabetes treatment. The clinical use of GLP-1 however, has been limited by short half life due to rapid degradation by dipeptidyl peptidase-IV (DPP-IV). The purpose of this study is to evaluate the potency and stability of newly designed GLP-1 analogs base on the amino acid sequence of Xenopus GLP-1, exendin-4, and human GIP (Glucose-dependent insulinotropic polypeptide).
Materials and Methods: The GLP-1 receptor is a member of G-protein-coupled receptor (GPCR) that induces the protein kinase A signaling pathway. The GLP-1 receptor cDNA was co-transfected into HEK293 with cAMP-dependent protein kinase A (PKA)-specific (CRE-luciferase) reporter. Various concentrations of chimeric GLP-1 agonists were treated to the cells for 6 hours and luciferase activities were determined. The stability of agonist was evaluated by determining the activity of the agonist that was preincubated in the presence of fetal bovine serum (FBS) which naturally contains DPP-IV activities.
Results: In this study, we observed GLP-1 was able to induce cAMP responsive element (CRE)-driven transcriptional activities. A chimeric GLP-1 analog with the sequence of Xenopus GLP-1 and exendin-4 (termed xGLP-E4) was as effective as exendin-4 (EC50 values of 0.17 nM, 0.11 nM, and 0.44 nM for xGLP-E4, Exendin-4, GLP-1, respectively). When incubated with FBS for 72 hours, the potency of GLP-1 was rapidly decreased to 15% of initial activity, but the activity of xGLP-E4 was maintained up to 85% of initial activity. The chimeric peptides with GLP-1 and GIP activated both the GLP-1 receptor and GIP receptors.
Conclusion: The addition of C-terminal ten amino acid sequences from exendin 4 to Xenopus GLP-1 may improve the potency to activate the GLP-1 receptor. Substitution of serine for alanine at the second position of the GLP-1 may improve the stability of the peptide against the action of DPP-IV. Combination of GIP and GLP-1 sequence allows activating both GLP-1 and GIP receptors.

0675

Pharmacological intervention against diabetic dyslipidaemia confers additive antidiabetic efficacy of insulinotropic GLP-1 analogue, liraglutide

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Background and Aims: Type 2 diabetes is frequently associated with secondary dyslipidaemia and hence with marked increased risk of cardiovascular disease.
Materials and Methods: We have used severely insulin resistant male Zucker Diabetic Fatty (ZDF) rats as a model for progressed human type 2 diabetes to assess therapeutic intervention with 42 days administration of the Glucagon-Like Peptide-1 (GLP-1) analogue liraglutide (L) and HMG-CoA inhibitor atorvastatin (A).
Results: Treatment with L either alone or in combination with A significantly reduced cumulated food intake with resulting loss of body weight, whilst A alone had no impact on feeding and body weight. Compared to vehicle, combination of L+A synergistically reduced fasting plasma glucose (21.4±2.1 vs. 29.5,±0.9 mmol/l). Long term glycaemic control as reflected by HbA1C was only marginally affected by L alone, whereas L+A significantly reduced HbA1C by 1.5% after 28 days of treatment (vehicle: 9.7±0.3%; liraglutide: 8.8±0.6%; atrovastatin: 9.2±0.2%; liraglutide+atorvastatin: 8.2±0.6*). At a terminal oral glucose tolerance test, treatment with A+L combination as well as treatment with liraglutide alone significantly improved glycaemic control. At day 34, 24-hour plasma glucose and insulin profiles were obtained by sampling every 4 hours. Both L+A and L alone significantly reduced 24-hour plasma glucose profile, whereas plasma insulin levels were significantly increased only with L+A treatment. Compared to vehicle, all treatment paradigms significantly lowered plasma levels of triglycerides and free fatty acids (vehicle: 0.81±0.05; liraglutide: 0.58±0.05**; atorvastatin: 0.65±0.04* liraglutide+atorvatstatin: 0.57±0.07** mmol/l). Plasma cholesterol was significantly lowered only by L+A treatment (vehicle: 4.5±0.3 mmol/l; liraglutide: 4.1±0.2 mmol/l; atorvastatin: 4.8±0.1 mmol/l; liraglutide+ atorvastatin: 3.9±0.2* mmol/l). It is concluded that therapeutic intervention against diabetes related secondary dyslipidaemia improves glycaemic control above and beyond therapeutic benefits of the insulinotropic actions of liraglutide.
Conclusion: As atorvastatin alone has no impact on glycaemic control, it can be speculated that be speculated that its therapeutic benefits are mediated indirectly via lowering lipotoxicity in insulin sensitive tissues.

PS 49

Inflammation and insulin resistance

0676

Signaling specificity of IL6 action on glucose and lipid metabolism in skeletal muscle

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Background and Aims: Physical exercise and muscle contraction leads to increased production and release of IL6 from skeletal muscle, and this has been suggested to enhance substrate metabolism and whole body glucose homeostasis. Our aims is to determine the effect of IL6 on glucose and fat metabolism.We hypothesize that PI3-kinase and AMPK pathways may play a role in IL6 action on muscle cell.
Materials and Methods: Primary human skeletal muscle cells were exposed to IL6 (25 ng/ml for 20 min to 8 days) and *AMPKa1/2si*RNA gene-silencing was applied to measure glucose and fat metabolism.
Results: Chronic IL6 exposure increased myotube fusion and formation and expression of *GLUT4*, *peroxisome proliferator activated receptor* (*PPAR*)α, *PPARδ*, *PPARγ*, *PPARγ coactivator 1*, *glycogen synthase*, *myocyte enhancer factor 2D, uncoupling protein* 2, *fatty acid transporter 4* and *IL6* ($p<0.05$), whereas *GLUT1*, *CEBPa* and *UCP3* was decreased. IL6 increased glucose incorporation to glycogen and fatty acid uptake and oxidation, concomitant with increased phosphorylation of AMP-activated protein kinase (AMPK), STAT3 and ERK1/2. IL6 also increased PI3 kinase activity (200%; $p<0.05$) which was blunted by subsequent insulin-stimulation (50%; $p<0.05$). IL6-mediated glucose metabolism was suppressed, but lipid metabolism was unaltered, by inhibition of PI3 kinase with LY294002. *si*RNA-directed depletion of AMPK reduced IL6-mediated fatty acid oxidation and palmitate uptake, but not glycogen synthesis.
Conclusion: IL6 increases glycogen synthesis via a PI3-kinase dependent mechanism and enhances lipid oxidation via an AMPK-dependent mechanism in skeletal muscle. Thus, IL6 directly promotes skeletal muscle differentiation and regulates muscle substrate utilization, promoting glycogen storage and lipid oxidation.
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0677

Inflammation is the primary signature of gene expression profile in leukocytes of insulin-resistant subjects

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Aims and Methods: Insulin resistance is a risk factor for diabetes, cardiovascular diseases (CVD) and metabolic syndrome, and is associated with systemic inflammation. To characterize the cellular phenotype of the inflammatory response related to insulin resistance, we performed a comprehensive evaluation of gene expression in circulating leukocytes of 53 subjects [28 insulin sensitive (IS) and 25 resistant (IR)] using a customized 22,000 features Agilent oligo-microarray. The study population was selected from a larger cohort in the first (IS) and third (IR) tertiles of 2-hours insulinemic response during OGTT. Enrolled subjects were free of diabetes, CVD and chronic inflammatory diseases, and the two groups were matched for age (mean±SD of IR vs IS: 60±5 vs 60±6 years), gender and whole body adiposity, assessed by BMI (26.7±2.8 vs 27.1±2.9 kg/m^2), waist (94.1±8.7 vs 95.2±9.6 cm), and total-body DXA (fat mass 30.3±7.0 vs 28.3±8.1%).
Results: Besides matching criteria, the 2 selected groups resulted also similar for blood pressure, total and LDL cholesterol, triglycerides, uric acids, and markers of subclinic systemic inflammation (white blood cells count, high-sensitivity C-Reactive Protein). Despite an equal clinical and laboratory phenotype, a total of 632 genes were significantly (>1.5 fold; fdr 2.5%) over-expressed in IR compared to IS, including genes of intracellular response to cytokines (IL1, 2, 4, 6, 18, and 30, chemokines, TNF-alpha and gamma-IFN), antigen processing and MHC activity, NFkB pathway activation, insulin metabolism (IRS1, MAPK family), and cell survival. A systematic classification of genes over-expressed in IR was performed on the basis of functional similarity using gene ontology (GO) annotation. EASE analysis on GO groups revealed significant (p for EASE score) activation of genes related to innate, as well as cell-mediated, immune response ["immune response" (<0.000), "defense response" (<0.000), "antigen processing" (<0.000), "response to external stimulus" (0.001), "antigen presentation" (0.001), "T-cell activation" (0.002), "lymphocyte activation" (0.003), "MHC class II receptor activity" (0.003), "immune cell activation" (0.003)] and insulin metabolism ["protein-tyrosine kinase activity" (<0.000), "protein serine/threonine kinase activity" (0.001)].

Conclusion: In apparently healthy subjects, the presence of insulin resistance is associated to significantly different gene expression profile in circulating leukocytes, being pro-inflammatory response and MAPK-pathway activation its primary expression signature. Inflammation associated to insulin resistance seems to involve both innate and cellular-mediated immunitary networks.

0678

Regulation of skeletal muscle chemokine receptor expression and its role in skeletal muscle insulin resistance

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Background and Aims: Adipocyte-conditioned medium (CM) contains a variety of chemokines that induce insulin resistance in skeletal muscle cells. Chemokines such as MCP-1 are also able to induce insulin resistance on their own. It has already been demonstrated that adipocytes express a wide range of chemokine receptors including CCR2, CCR4 and CCR10. However, the expression of chemokine receptors in skeletal muscle where chemokines might play a role in the development of diabetes was not yet established. In addition, this study aimed to analyse the regulation of these receptors in the insulin resistant state in human skeletal muscle cells.
Materials and Methods: Expression of chemokine receptors in human skeletal muscle cells and adipocytes was analysed by RT-PCR and Western Blot. Differentiated skeletal muscle cells were incubated for 24 h, 48 h und 72 h with high glucose/high insulin (GI) or TNFα in order to induce insulin resistance. In addition to these treatment regimens, skeletal muscle cells were co-stimulated with TNFα and MCP-1 or CM for 24 and 48 h to analyse possible synergistic effects of different situations of skeletal muscle insulin resistance.
Results: Human skeletal muscle cells and adipocytes expressed the CC chemokine receptors CCR1/2/4/5/10 and the CXC chemokine receptors CXCR1/2. While the expression at the protein level of CCR4 and CCR10 was similar in muscle cells and adipocytes the expression of CCR2 was significantly higher in skeletal muscle cells. Treatment of skeletal muscle cells with GI induced insulin resistance on the level of Akt and GSK3α/β as soon as after 24 h. TNFα reduced insulin-stimulated Akt phosphorylation in myotubes after 48 h and 72 h without having any effect on GSK3 phosphorylation. Treatment with CM and MCP-1 also lead to skeletal muscle insulin resistance, that could however not be increased by co-stimulation with TNFα. CCR2 was downregulated by TNFα (24 h) and MCP-1 (48 h) and upregulated by GI (24–72 h) and TNFα (72 h). CCR4 was decreased after treatment with MCP-1 (48 h) and TNFa (72 h). MCP-1 (24–48 h) and TNFα (48 h) also downregulated the expression of CCR10.
Conclusion: Human skeletal muscle cells and adipocytes express different chemokine receptors that may play a role in the negative crosstalk between adipose tissue and skeletal muscle and may be involved in the development of skeletal muscle insulin resistance by mediating adipocytokine-induced insulin resistance. CCR2 is highly expressed in skeletal muscle cells compared to adipocytes possibly explaining the prominent sensitivity of skeletal muscle cells towards MCP-1. Upregulation of CCR2 by high glucose/high insulin and TNFα may sensitize human skeletal muscle cells towards adipocytokine-induced insulin resistance.

0679

Elevated serum monocyte chemoattractant protein-4 (MCP-4) levels in overweight subjects and its correlation with chronic inflammation

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Background and Aims: Chronic inflammation observed in obesity has been reported to be implicated in the development of atherosclerosis; however its mechanism has not been fully elucidated. We screened candidate chemokines which link chronic inflammation and obesity.
Materials and Methods: Japanese overweight (n=39, BMI 28.7±0.65 kg/m^2) and normal weight subjects (n=24, BMI 22.3±0.45 kg/m^2) were enrolled into cross-sectional study. Using antibody-based protein microarray, spot intensities of monocyte chemoattractant protein-4 (MCP-4), eotaxin and eotaxin-2 correlated with anthropometric parameters. We further measured serum concentration of these chemokines and mRNA levels in adipose tissues obtained from volunteers.
Results: Serum MCP-4 levels showed positive correlation with BMI (r=0.318, p=0.014), waist (r=0.316, p=0.018) and waist hip ratio (r=0.264, p=0.049). Furthermore, MCP-4 correlated with HOMA-IR (r=0.392, p=0.002) and high-sensitivity C-reactive protein (hsCRP) (r=0.350, p=0.006), and HDL cholesterol (r=−0.409, p=0.001). In stepwise multiple regression analyses, hsCRP independently correlated with MCP-4 levels. Using real time RT-PCR, the

expression of MCP-4 mRNA in visceral adipose tissue positively correlates with BMI. Serum eotaxin levels correlate with BMI (r=0.262, p=0.045) and waist hip ratio (r=0.383, p=0.003). Serum eotaxin-2 levels correlated with BMI (r=0.464, p<0.001), waist (r=0.333, p=0.017) and waist hip ratio (r=0.278, p=0.048). However eotaxin and eotaxin-2 levels did not show significant correlation with hsCRP.

Conclusion: Serum levels of MCP-4, eotaxin and eotaxin-2, which belong to CC chemokine family and share CC chemokine receptor 3, correlated with BMI. MCP-4 further correlated with degree of chronic inflammation. These chemokines, especially MCP-4, may be critical molecules which link obesity and chronic inflammation.

0680

Tumour necrosis factor (TNF)alfa and interleukin (IL)-6 impair differentiation of preadipocytes and promote an inflammatory phenotype

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Background and Aims: Obesity is a central and causal part of insulin resistance and type 2 diabetes. Enlargement of adipocytes induces inflammation and macrophage recruitment into the adipose tissue with production of a variety of inflammatory cytokines such as interleukin (IL)-6, TNFalfa, plasminogen activator inhibitor (PAI)-1 and monocyte chemoattractant protein (MCP)-1. These agents can exert local effects in the adipose tissue or systemic effects in other tissues such as liver and muscle. Both IL-6 and TNFalfa have been shown to impair adipocyte differentiation of 3T3-L1 preadipocytes. In this study, we examined particularly how TNFalfa prevents preadipocyte differentiation.

Materials and Methods: 3T3-L1 preadipocytes were incubated with or without TNFalfa (1.5 ng/mL) or IL-6 (20 ng/mL) from the induction of differentiation and for 8 days. RNA was extracted and analysed with real-time PCR and immunoblots were performed on cell lysates.

Results: Both IL-6, and in particular, TNFalfa prevented preadipocyte differentiation and promoted an inflammatory phenotype. IL-6 did not prevent the initiation of differentiation but prevented the cells from entering the terminal differentiation through maintaining the Wnt signaling with low levels of axin and increased levels of beta-catenin. TNFalfa did not prevent the induction of either C/EBPbeta or C/EBPdelta nor the activation of the cAMP response element binding protein (CREB). During induction of differentiation, the inhibitory C/EBP homologue protein (CHOP)-10 is temporarily induced but this protein was not affected by TNFalfa and was rapidly down-regulated. The cyclin D kinase (CDK) inhibitor, p27, was also degradaded in the presence of TNFalfa and this is necessary for the cells to normally enter the S-phase during mitotic clonal expansion. However, TNFalfa maintained the Retinoblastoma protein (pRb) during differentiation and totally prevented the induction of PPARgamma and C/EBPalfa. In addition, TNFalfa induced the expression of GATA2, which is a critical transcription factor that can suppress adipogenesis and promote an inflammatory phenotype of the cells.

Conclusion: These data show that the presence of TNFalfa does not alter the early induction of differentiation with a decrease in CHOP-10 and p27 expression, induction of C/EBPbeta and C/EBPdelta and activation of CREB. However, TNFalfa totally prevented induction of PPARgamma and C/EBPalfa and, thereby, differentiation towards an adipocyte phenotype and, instead, induced an inflammatory phenotype. The induction of GATA2 by TNFalfa is likely to be an important mediator of the altered phenotype of the cells.

0681

CD166 (ALCAM) modulates inflammatory responses upon RAGE-ligand activation *in vivo*

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Background and Aims: RAGE is a multiligand receptor known to play a role in chronic inflammatory diseases. Deletion of RAGE provided protection from the lethal effects of septic shock caused by cecal ligation and puncture (CLP), while it did not protect from inflammatory reactions in delayed type hypersensitivity (DTH). DTH, however, could be blocked by sRAGE in both, WT and RAGE-/--mice, implying that other receptors binding RAGE-ligands might compensate for RAGE-deficiency. ALCAM (CD166) shares significant structural homology to RAGE. This implies that ALCAM is a candidate receptor able to bind one or more of RAGE ligands.

We studied whether ALCAM is a receptor able to bind RAGE ligands and compensate for RAGE deficiency in settings of adaptive immune response. Therefore, we identify the effects of sALCAM and ALCAM neutralizing antibodies in the delayed type hypersensitivity (DTH) model in wild type (WT) and RAGE -/-- mice.

Materials and Methods: 8 weeks old male WT and RAGE-/-- mice were sensitized by injection of 100 µl 25 mg/ml methylated BSA (mBSA), 50 mg/ml Dextran, 50% Freunds incomplete adjuvant, 0.9% NaCl over the left

inguinal lymph node. 21 days later DTH reaction was induced (footpad injection 50 μl 0.25 mg/ml mBSA). sRAGE, sALCAM and ALCAM antibody were applied i.p. (100 μg/mouse) 6 h before and 6 and 12 h after mBSA injection. 24 h after footpad injection mice were sacrificed and the footpads examined morphologically for signs of swelling and inflammation.

Results: In vitro studies in CHO cells overexpressing ALCAM confirmed that treatment with the RAGE-ligand S100B led to activation of transcription factor NF-κB. That effect was reversed by administration of both, sRAGE and sALCAM. In addition, RAGE-ligand induced NF-κB binding activity in S100B stimulated RAGE -/- - fibroblasts was inhibited by sRAGE, sALCAM and ALCAM neutralizing antibodies (ab) While administration of sRAGE led to significant decrease of inflammation in the footpads of both WT and RAGE -/- - animals, sALCAM and ALCAM neutralizing antibodies reduced inflammation only in RAGE -/- -, but not in WT mice. Furthermore, ALCAM ab and sALCAM treatment led to the reduction of expression of TNFα, IL-1β, IL-6 and IFNγ mRNA in the footpad homogenates of RAGE -/- - animals.

Conclusion: ALCAM is a RAGE homologue receptor that modulates inflammatory responses upon RAGE-ligand activation *in vivo*.

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Relationships between serum adiponectin, IL-10 and IL-18 concentrations and muscle lipid fractions in healthy humans

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Background and Aims: Intramuscular lipids, including ceramide, might be responsible for the development of insulin resistance. Numerous studies also indicate, that insulin action might be inversely associated with circulating proinflammatory cytokines, like IL-18 and positively related to antiinflammatory factors, like adiponectin and IL-10. Interrelationships between the above circulating factors and intramuscular lipids remain unclear. The aim of the present study was to estimate the relationships between serum adiponectin, IL-10 and IL-18 concentrations and muscle lipid fractions in healthy humans.

Materials and Methods: The study group consisted of 37 male subjects with normal glucose tolerance, without morbid obesity or other serious medical problems. Euglycemic hyperinsulinemic clamp and a biopsy of vastus lateralis muscle were performed. To avoid contamination of extracellular fat, muscles were lyophilized. After lipid extraction, ceramides were hydrolyzed and then were estimated as the sphingosine content with HPLC system. Muscle free sphingosine and sphinganine conent and the activities of the enzymes: neutral and acid sphingomyelinase, neutral and alkaline ceramidase and serine palmitoyltransferase was also measured. Muscle free fatty acid (FFA), diacylglycerol (DAG) and triacylglycerol (TG) were separated with thin-layer chromatography. The content of particular fatty acids in these fractions was determined by gas-liquid chromatography.

Results: Insulin sensitivity was related to circulating cytokines (adiponectin, r=0.38, p=0.021; IL-10, r=0.47, p=0.0034; IL-18, r=−0.37, p=0.023) and to muscle lipids (ceramide, r=−0.45, p=0.024; DAG, r=−0.43, p=0.031; TG, r=−0.52; p=0.01). It was also associated with the activities of the enzymes regulating ceramide metabolism (serine palmitoyltransferase, r=−0.58, p=0.002; alkaline ceramidase, r=−0.37, p=0.025). The relationship between insulin action and neutral ceramidase activity approached the level of significance (r=−0.32, p=0.056). Adiponectin was negatively related to muscle ceramide content (r=−0.44, p=0.027) and to serine palmitoyltransferase activity (r=−0.35, p=0.032). IL-10 and IL-18 were associated, in an opposite manner, with muscle DAG (IL-10, r=−0.46, p=0.022; IL-18, r=0.40, p=0.049) and muscle TG (IL-10, r=−0.50, p=0.014; IL-18, r=0.46, p=0.026). IL-10 was also related to muscle FFA pool (r=−0.51, p=0.026).

Conclusion: There are multiple associations between circulating cytokines and muscle lipid pool, which possibly might influence insulin sensitivity. Adiponectin is related to muscle ceramide content, mostly through an association with de novo synthesis pathway (as indicated by serine palmitoyltransferase activity). In contrast, IL-10 and IL-18 are associated with muscle FFA-DAG-TG pathway.

0683

Iron overload in type 2 diabetes and its relationship to chronic inflammatory process

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Background and Aims: Iron overload has been implicated in the etiopathogenesis of diabetes mellitus, but limitations of the existing markers and involvement of confounding variables have made it difficult to ascertain its precise role in diabetes. Chronic subclinical inflammation is claimed to be present in both diabetes and iron overload is. The objective of the present study was to explore the body iron status and chronic inflammation in type 2 diabetic subjects and also to investigate the interrelationship between these two variables in the context of the hyperglycemic state.
Materials and Methods: Eighty-nine diabetic subjects as case and 54 healthy individuals as control, matched by BMI [mean±SD (kg/m2: 25±4.62 in diabetic vs 24.43±4.01 in control, p<0.451, 95% CI −2.07−0.93)] were included in the study. Serum ferritin and serum sTfR were used as markers of body iron status and those were measured by MEIA and ELISA methods respectively. Serum C-reactive protein (CRP) was used as a marker of inflammation and it was estimated by FPIA technology. Serum fasting glucose and lipid profile were measured by enzymatic-colorimetric method.
Results: The diabetic subjects had higher systolic blood pressure [mean±SD (mmHg: 127±12.57 in diabetic vs120± 4.86 in control; p<0.001, 95% CI −9.06–3.17)], fasting glucose [median (range): {mmol/l: 8.72 (4.12–26.96) in diabetic vs 4.98 (3.71–6) in control; p<0.001}] and serum triglyceride levels [median (range): {mg/dl: 164.5 (59.98–801) in diabetic vs 121 (50–276) in control; p<0.001}] when compared to control. The diabetic subjects had higher serum ferritin levels than the control subjects [median (range): {ng/ml: 103 (28.7–605.3) in diabetic vs 73.47 (12.18–189) in control; P<0.019}]. However, no difference in sTfR concentrations were observed between diabetic and control subjects [median (range): {nmol/l: 21.6 (10.3–44.3) in diabetic vs. 19.76 (13.58–38.89) in control, P<0.151}]. Regarding CRP level, the diabetic subjects showed considerably higher value when compared to control subjects [median (range): {mg/dl: 1.35 (0–3.68) in diabetic vs 0 (0–1.48) in control, p<0.001}]. In control subjects log-sTfR levels showed significant negative correlation with log-ferritin (r=−0.42, p<0.001). In contrast diabetic subjects (r=0.033, p<0.755) did not show any significant association. Significant positive correlation was observed between log-ferritin and CRP in diabetic subjects (r=0.254; p<0.016), but in the control subjects no association was found (r=0.04, p<0.78). In diabetic subjects fasting glucose level was not significantly associated with log-ferritin (p<0.59) and CRP levels (p<0.22). In multiple regression analysis, an independent inverse relationship between log-ferritin and log-sTfR was observed in controls (p<0.001, 95% CI −2.26–0.74). However, there was no such relationship between log-ferritin and log-sTfR in diabetic subjects. There was a positive association of log-ferritin with CRP in the diabetic subjects (p<0.053).
Conclusion: The present study demonstrates that (a) body iron store is elevated in type 2 diabetes mellitus; (b) diabetes is associated with a subclinical chronic inflammatory process; (c) hyperglycemia does not have a straightforward relationship with iron overload or with inflammation; and (d) chronic inflammation is associated with excess body iron store in diabetes.
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0684

Plasma CD36 is related to obesity and low grade inflammation

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Background and Aims: Recently we have demonstrated the presence of soluble CD36 (sCD36) in cell free plasma, and in a small population of type 2 diabetic patients we found markedly increased levels of sCD36. Furthermore, sCD36 was tightly correlated with insulin resistance and BMI. We aimed to evaluate if this relationship was also present in a larger population of glucose intolerant males, and furthermore to study the relationship between markers of low grade inflammation and sCD36.
Materials and Methods: 147 apparently healthy men, 90 normotolerant and 57 glucose-intolerant subjects were included in the study. Insulin sensitivity was assessed by the minimal model, and sCD36 was measured by ELISA.
Results: When all subjects were considered as a whole, circulating sCD36 concentration was significantly associated with fat-free mass, glycosylated hemoglobin, plasma triglycerides, platelet number, plasma interleukin 6 (IL-6) and with glucose effectiveness (p between 0.04 and 0.001). However, the association with fat-free mass, triglycerides, platelet number and IL-6 was mainly observed in subjects with glucose intolerance but not in normotolerant subjects. In subjects with glucose intolerance, sCD36 was positively associated with fasting glucose and negatively with insulin sensitivity.
In a multiple linear regression analysis to predict circulating sCD36, among subjects with glucose intolerance, IL-6 (p=0.0035) and fat mass (p=0.025) were independently associated with plasma sCD36 concentration, contributing to 12% and 9%, respectively, of sCD36 variance after

adjusting for age, fasting glucose, plasma triglycerides, platelet count and insulin sensitivity.
Conclusion: We propose that levels of sCD36 in men reflect obesity related low grade inflammation and insulin resistance.
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0685

Increased circulatory metalloproteinase-2 and metalloproteinase-9 levels and activities in patients with type 1 diabetes mellitus

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Background and Aims: Type 1 diabetes mellitus (T1DM) is an autoimmune disorder with unknown etiology. The incidence of T1DM in Taiwanese is among the lowest range of diabetic incidence worldwide. Metalloproteinases (MMPs) are suggested to play important roles in the development and complications of autoimmune disorders. The present study is to examine if the MMPs expression or activities are altered in Taiwanese T1DM patients.
Materials and Methods: Blood samples were collected from 201 T1DM and 189 non-diabetic control subjects in Division of Endocrinology & Metabolism, Veterans General Hospital-Taipei. Levels and activities of plasma MMP-2 and MMP-9 in T1DM patients were investigated and compared to those of control subjects by enzyme-linked immunosorbant assay and zymorgraphy, respectively. The demographic characteristics and clinical manifestations of each patient were filed for statistical analysis. Written consent was obtained from all the study subjects after the nature of the procedure was explained. The study has been approved by the Ethics Committee of Chung Shan University Hospital.
Results: The zymagraphy data showed that both MMP-2 and MMP-9 activities in sera from patients with T1DM were significantly higher than that from control subjects. When the MMP-2 and MMP-9 expression levels were quantitatively measured by ELISA, MMP-2 level in control subjects and T1DM patients was 146.1±34.2 and 241.5±73.7 *n*g/mL, respectively. The MMP-9 level in control subjects and T1DM patients was 51.4±57.1 and 305.3±180.6 *n*g/mL, respectively. Both MMP-2 and MMP-9 levels were significantly higher in sera of T1DM patients, compared to that from control subjects. No significant correlation between MMPs levels or activities with patients' demographic or clinical data was found.
Conclusions: MMPs expression and activities are significantly increased in patients with T1DM. Our results not only document plasma MMPs levels and activities in Taiwanese population with possibly the lowest type 1 diabetic incidence, but also suggest that MMPs expression and activity are elevated before the onset of complications in diabetic patients.

PS 50

Metabolic effects of adipocytokines

0686

Pattern of expression of adiponectin receptors in human adipose tissue depots and its relation to metabolic state

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Background and Aims: The adiponectin receptors, APMR1 and APMR2, are thought to mediate the insulin-sensitizing effects of adiponectin. We examined whether Adipo R1 and R2 expression in human subcutaneous (SAT) and visceral (VAT) adipose tissue correlates with adiponectin gene (APM1) expression and *in vivo* metabolic parameters in morbid obesity with or without diabetes.
Materials and Methods: Total RNA was extracted from SAT and VAT tissue obtained during abdominal surgery from 7 lean non-diabetic women (C) (60±10 years, BMI 25.1±3.1 kg/m2), 15 obese diabetic women (DM2-Ob) (49±9 years, BMI 45.0±7.5 kg/m2) and 30 obese women with normal glucose tolerance (Ob) (38.6±11 years, BMI 47.6±8.1 kg/m2). cDNA was synthesised and relative quantifications of adiponectin receptors mRNA (target gene) was performed by Real-Time PCR. Results were expressed as the target to reference (hypoxantine-phosphorybosyltransferase) ratio for each sample.
Results: In SAT, APMR1 was significantly more expressed in C than Ob subjects (1.14±0.74 vs 0.60±0.50 vs 0.90±0.72, respectively, p=0.04 for C vs Ob). Likewise, APMR2 was more expressed in C compared to Ob or DM2-Ob (11.6±8.1 vs 5.8±6.1 and 5.8±5.5, p≤0.05). In VAT, APMR1 was more abundantly expressed in C than DM2-Ob (1.1±0.8 vs 0.7±0.4 and 0.6±0.3, p=0.02). Furthermore, APMR2 was again more expressed in C than DM2-Ob (1.1±1.0 vs 0.9±0.8 and 0.6±0.5, p=0.05). A

positive correlation was found between APMR1 and APMR2 expression in SAT ($p \leq 0.0001$, $r^2=0.25$) as well as VAT ($p \leq 0.0001$, $r^2=0.76$), but in SAT APMR2 was about ten times higher than APMR1. APMR1 expression in SAT was inversely related to BMI ($r^2=0.10$, $p=0.02$) and serum HDL-cholesterol ($r^2=0.10$, $p=0.04$). Both APMR1 and APMR2 expression in VAT were strongly related to fasting plasma insulin ($r^2=0.16$, $p=0.015$ and $r^2=0.14$, $p=0.028$, respectively) and serum triglycerides ($r^2=0.17$, $p=0.007$ and $r^2=0.16$, $p=0.009$, respectively). In both SAT and VAT, a direct correlation was found between the expression of either receptor and APM1 expression.

Conclusion: In conclusion, severe obesity is associated with a lower expression of both APMR1 and APMR2 in SAT, whereas expression of both receptors in VAT is reduced only when diabetes were present. The pattern of association with the clinical variables suggests that, in SAT APMR receptor downregulation (predominantly APMR2) is related to body fat mass, while in VAT it is linked with hyperglycaemia, hyperinsulinaemia and dyslipidaemia.

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Adiponectin and insulin resistance modulate the gene expression of adiponectin receptors in human adipose tissue

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Background and Aims: Two adiponectin (AD) receptors have been described, ADIPOR1 ubiquitously expressed and ADIPOR2 more expressed in liver and skeletal muscle. Their putative role as insulin sensitizer has been proved in rodents but it is uncertain in humans. The gene expression of ADIPORs in human adipose tissue and its relation with adiposity and insulin sensitivity has been poorly studied. We investigated 1) the mRNA expression of AD, ADIPOR1 and ADIPOR2 in subcutaneous (SAT) and visceral adipose tissue (VAT), 2) their relation with adiposity, AD levels in culture medium and serum and with circulating levels of insulin, fasting and post load glucose and lipids.

Materials and Methods: Abdominal SAT was obtained in 60 obese subjects (86% women, 44.7±11.2 yr, BMI 37.6±5.1 kg/m^2, waist 112.1±13.1 cm). 11/60 subjects underwent bariatric surgery and VAT was also taken. ADIPO and ADIPORs mRNA was measured using RT-PCR. Insulin resistance was defined with HOMA-IR ≥2.5. AD release was measured from SAT and VAT minced and placed into culture medium for 24 h.

Results: Family history of diabetes did not influence the expression of AD and ADIPORs. The expression levels of AD and ADIPORs were lower in men than in women ($p=0.05$). We therefore analysed the women only. In 52 obese women, ADIPOR2 expression was 4-fold higher than that of ADIPOR1 (2.0±1.1 vs 0.4±0.3 in SAT and 2.0±0.7 vs 0.5±0.3 in VAT, $p<0.0001$ for both). The mRNA expression levels of ADIPORs were highly correlated with each other (r 0.781, $p<0.0001$ in SAT and r 0.541, 0.05 in VAT) and in SAT with that of AD (r 0.816, $p<0.0001$ for R1 and r 0.890, $p<0.0001$ for R2). The gene expression of AD and ADIPOR2 was not related to AD release from SAT and VAT. The SAT expression of AD and ADIPOR2 was positively correlated with serum AD (r 0.274, $p<0.05$ and r 0.288, $p<0.05$) and negatively with waist (r −0.552, $p<0.0001$ and r −0.558, $p<0.0001$), insulin (r −0.341, $p<0.05$ and r −0.283, $p<0.05$) and HOMA-IR (r −0.321, $p<0.05$ and r −0.265 $p<0.05$). In the multivariate analysis with ADIPOR2 as dependent variable, only AD remained positively and independently correlated with ADIPOR2 (β 0.895, $p<0.0001$). 48% of women had insulin resistance. These women compared to those without insulin resistance had significantly lower levels of serum AD (4.4±1.9 vs 6.3±2.5, $p<0.01$) and lower expression levels in SAT of AD (15.2±6.8 vs 23.1±14.3, $p<0.05$) and ADIPOR2 (1.7±0.8 vs 2.3±1.3, $p<0.05$). Low HDL and high triglycerides were associated with lower levels of serum AD, but not of AD and ADIPOR2 expression in adipose tissue.

Conclusion: 1) Human adipose tissue express more abundantly ADIPOR2 than ADIPOR1, 2) The strict relation between AD and ADIPORs expression suggests autocrine/paracrine effects for AD in the adipose tissue, 3) Serum AD reflects the levels of expression of AD in SAT, 4) AD and ADIPOR2 expression in SAT as well as serum AD are negatively affected by insulin resistance.

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Adiponectin does not mediate obesity-related insulin resistance in young children: a longitudinal study

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Background and Aims: Adiponectin, an adipokine secreted exclusively by adipose tissue, is present in abundant concentrations in the circulation. It has both insulin sensitising and anti-atherogenic properties. In adults,

adiponectin has independent inverse associations with obesity and hyperinsulinaemia. Cross-sectional studies of predominantly obese, pubertal individuals indicate that adiponectin may also play an important role in glucose regulation in children. To date, limited data are available in healthy young children. Our aim was to characterise adiponectin levels in prepubertal children and their relationship to BMI, body fat and insulin resistance over time.
Materials and Methods: Participants: healthy children (114 boys, 86 girls) from stratified randomly-selected schools enrolled in The EarlyBird Study measured at 5,6,7 and 8 years. Measures: adiponectin, insulin resistance (HOMA-IR), BMI, adiposity measured by sum of 5 skinfolds (SSF) (correlation with total body fat measured by DEXA r=0.9, p<0.001 in the children aged 7 and 8 years).
Results:
1. Both BMI sds and SSF rose progressively from age 5 to 8 y (+0.1 sd and+30%, p<0.001).
2. HOMA-IR nevertheless fell progressively (−24%, p<0.001).
3. Adiponectin levels correlated strongly year-on-year (r=0.71 to 0.79, p<0.001).
4. In boys, adiponectin fell 11.1% (p<0.001) from 5 y-8 y (13.8–12.9–12.7–12.5 ug/ml), and in girls it fell 6.5% (p=0.02) (13.7–12.6–13.2–12.6 ug/ml).
5. HOMA-IR was directly related to BMI at all ages (r=0.25 to 0.47, p<0.001) and to SSF from age 6 in girls and age 7 in boys (r=0.22 to 0.55, p<0.05).
6. Adiponectin did not correlate with BMI at any age, and with SSF only weakly 8 year old girls (r=−0.22, p<0.05).
7. There was no correlation of adiponectin with HOMA-IR in girls of any age, and in boys only at 5 and 6 y (both r=−0.19, p<0.05).
Conclusion: Adiponectin rank order was maintained from year to year (suggesting the measure was precise and the levels systematic), and it fell (as expected) as adiposity rose. The behaviour of insulin resistance in the face of rising adiposity was unexpected. Insulin resistance and adiposity were correlated, as expected**,** but the relationships of adiponectin with adiposity and insulin resistance were weak and inconsistent. We found little evidence that circulating adiponectin mediates obesity-related insulin resistance in young children, although relationships may become apparent during the pubertal years.
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0689

Low adiponectin and high resistin plasma levels predict 5-year mortality after first-ever ischaemic stroke in type 2 diabetic patients

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Background and Aims: The adipocytokines resistin (RS) and adiponectin (ADPN) are implicated - by contradictory actions - in insulin sensitivity, inflammation and cardiovascular disease. Data on the prognostic significance of these proteins in diabetic patients with cerebrovascular events are lacking.The aim of the present study was to investigate the association between i) plasma RS and ADPN levels and ii) stroke severity and long-term outcome in type 2 diabetic patients with first-ever ischemic stroke.
Materials and Methods: RS, ADPN and C-reactive protein (CRP) in the first 24 hours after the onset of stroke were measured by sandwich ELISA. Initial infarct volume and clinical severity were assessed by brain CT and the National Institute of Health Stroke Scale (NIHSS), respectively, whereas insulin sensitivity was estimated using the homeostasis model assessment (HOMA). A multivariate Cox's proportional hazards model was used to evaluate 5-year survival.
Results: Data from 60 individuals were analyzed (mean age 67.1±10.4 years, body mass index [BMI] 27.1±5.3 kg/m^2, 32 men, mean HbA_{1c} 8.1%, fasting glucose 168 mg/dl, fasting insulin 19.2 μU/ml, HOMA 8.2). Twenty-eight deaths (46.7%) occurred within 5 years after stroke. Mean ADPN and RS values were 5.8±3.1 μg/ml and 28.3±5.9 ng/ml, while median values of infarct volume, NIHSS score and CRP were 45 (interquartile range 9–108) cm^3, 15 (6–30) and 10 (4–17) mg/L, respectively. There was a significant inverse correlation of ADPN with RS (r=−0.72; p<0.001), CRP (r=−0.61; p<0.001), BMI (r=−0.39; p<0.05), mean arterial pressure (r=−0.43; p<0.05), infarct volume (r=−0.34; p<0.05) and NIHSS score (r=−0.41; p<0.05). RS showed a negative association with HDL-cholesterol (r=−0.33; p<0.05), whereas it was positively related to CRP (r=0.61; p<0.01), BMI (r=0.59; p<0.01), fasting insulin (r=0.38; p<0.05), fasting glucose (r=0.42; p<0.05) and HOMA (r=0.55; p<0.01). In multivariate analysis, the risk of death within 5 years after index stroke was significantly associated with the presence of

coronary heart disease (hazard ratio [HR] 8.7, 95% confidence intervals [CI] 3.6, 20.4), NIHSS score in the highest tertile (HR 7.1, 95% CI 3.2, 18.4), CRP in the highest tertile (HR 5.6, 95% CI 3.1, 16.2), age>70 years (HR 4.4, 95% CI 2.2, 14.3), ADPN in the lowest tertile (HR 3.5, 95% CI 1.5, 9.8) and RS in the highest tertile (HR 2.1, 95% CI 1.2, 6.9).

Conclusion: Low ADPN and high RS plasma levels on admission of first-ever ischemic stroke in type 2 diabetic patients appear to reflect the extent of brain injury, the stroke severity and the risk of 5-year mortality, independently of the impact of acute phase reaction as estimated by CRP concentration. The above findings provide additional support to the view that there is an adipo-vascular axis linking insulin resistance, obesity and atherosclerosis.

0690

Serum adipocytokine visfatin at first trimester is an early predictor of the insulin sensitivity index (ISI) of the second trimester in normal pregnancy

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Background and Aims: Adipose tissue is an active endocrine organ producing adipocytokines and is involved in glucose metabolism, inflammation, and cardiovascular disease. Throughout pregnancy there is an increase and redistribution of fat deposition. During the second trimester of pregnancy the regulatory effects of insulin on fat and carbohydrate metabolism become impaired so that a state of relative insulin resistance occurs. Recently it has been found adipocytokine visfatin to be expressed and secreted from splachnic fat in humans.

Aim of this study is to examine the role of adipocytokines in the process of insulin resistance in normal pregnancy.

Materials and Methods: 40 pregnant women with mean BMI (mean+SD) 24.7+4.2 kg/m2 before pregnancy, and age of 29.5+4.5 years, with no history of type 2 Diabetes Mellitus were followed. After informed consent pregnant women did 3 visits between 10th and 12th week, 24th to 26th and 35th με 36th where a single observer did anthropometric measurements (weight, height), and a 75 gr OGTT with blood serum collection at 0, 5, 15, 30, 60, 90 και 120 minutes. From the above tests the insulin sensitivity index (ISI) and HOMA-R (insulin resistance index) were estimated during each trimester. None of the above pregnant women developed Gestational Diabetes during pregnancy.

Results: During analysis with ANOVA-one way there was a statistically significant difference in the change of maternal weight between 1st and 2nd trimester (p=0.005) and between 2nd and 3rd (p=0.0005). ISI index was statistically significant decreased between 2nd and 3rd trimester (p=0.02) but also between 1st and 3rd. HOMA-R showed statistically significant difference between 1st and 3rd trimester (p=0.03). Forward multiple regression analysis showed first trimester serum visfatin levels (beta=1.01, p=0.01) as the only predictor of 2nd trimester ISI among other metabolic parameters of first trimester like adiponectin, hs-CRP, IL-6, leptin, maternal weight. Finally the main predictors of serum visfatin levels during 2nd trimester were adiponectin (beta=−0.81, p=0.01) basal insulin (beta=−1.55 p=0.02) and maternal weight (beta=1.98 p=0.03).

Conclusion: The study shows for the first time that adipocytokine visfatin has a role in insulin sensitivity in normal pregnancy in contrast with other adipocytokines produced by adipose tissue (adiponectin, hsCRP, leptin, IL-6). Its correlation with insulin at 0 min confirms its possible insulinmimetic action.

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Effect of acute hyperinsulinaemia with and without AT-1 receptor blockade on plasma concentrations of leptin and adiponectin and their expressions in adipose tissue in diabetes mellitus

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Background and Aims: Recently it was suggested, that AT-1 receptor blockers (ARB) have distinct antidiabetic and insulin-sensitising properties, however the underlying mechanisms are not clear. The effect of ARBs on leptin and adiponectin that are involved in insulin resistance, has not been clarified in humans till now.

The aims of our study were to measure the plasma concentrations of leptin and adiponectin and their expressions in abdominal subcutaneous adipose tissue and to test their response to acutely-induced hyperinsulinaemia with and without acute AT-1 receptor blockade in type 2 diabetes (D) and in healthy subjects (H).

Materials and Methods: Plasma concentrations of leptin and adiponectin were measured: 1) before (0 min), at 30 min and 240 min of hyperinsulinaemic (1 $mU.kg^{-1}.min^{-1}$) euglycaemic (5 $mmol.l^{-1}$) clamp (HIC), and 2) during HIC after acute AT-1 receptor blockade (losartan cumulative dose 200 mg) (AT-HIC) using the same protocol, in 11 D and 8 H. Needle biopsy of abdominal subcutaneous adipose tissue was performed before (0 min), at 30 min and 240 min of both clamps to asses the relative expressions of leptin and adiponectin using the real-time PCR method (expression of adipokine mRNA relative to cyclophilin mRNA).
Results: Insulin sensitivity, expressed as metabolic clearence rate of glucose, was significantly higher in H compared to D ($p<0.001$), without any changes during AT-HIC in both groups. Plasma concentrations of leptin and adiponectin were comparable during HIC and AT-HIC in both groups and were not affected by insulin. The relative expressions of adiponectin in abdominal subcutaneous adipose tissue were higher after losartan administration in D, but not in H (see the table; $p<0.05$; ANOVA), without any dynamic changes during HIC and AT-HIC in both groups. The expressions of leptin were not changed by hyperinsulinaemia with or without losartan administration both in D and H.
Conclusion: In type 2 diabetes and healthy subjects, acute hyperinsulinaemia does not influence plasma levels and adipose tissue expressions of adiponectin and leptin.
In type 2 diabetes, but not in healthy subjects, losartan administration stimulates the expression of adiponectin in subcutaneous adipose tissue, leptin expression is not affected. The results support the hypothesis, that the changes in adiponectin expression might be involved in the improvement of insulin sensitivity connected with ARB treatment.

Relative expressions of adiponectin (AdipoQ mRNA/HCYC mRNA)

	Type 2 diabetes			Healthy subjects		
	0 min	30 min	240 min	0 min	30 min	240 min
HIC	43.97± 20.03	50.91± 18.17	45.72± 15.71	23.99± 5.70	31.03± 9.13	40.82± 11.15
AT-HIC	55.14± 24.74	53.09± 19.90	54.07± 13.63	43.92± 22.91	31.67± 3.83	28.26± 7.74

Data are expressed as mean±standard error.
For type 2 diabetes: HIC vs. AT-HIC: $p<0.05$; ANOVA.

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0692

Increased oxidative stress and decreased plasma levels of adiponectin are associated with insulin resistance in Japanese metabolically obese, normal-weight men

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Background and Aims: Recently, we described metabolically obese, normal-weight (MONW) subjects [BMI <25 kg/m^2 and visceral fat area (VFA) evaluated by abdominal computed CT scanning≥100 cm^2] in the Japanese population, and demonstrated that insulin resistance together with visceral fat accumulation play an important role in the pathophysiology of metabolic obesity. The roles of oxidative stress and adiponectin in the pathophysiology of obesity have been the focus of many investigations. Oxidative stress is significantly correlated with decreased plasma levels of adiponectin in subjects with obesity, whereas both of them are significantly correlated with insulin resistance. However, the relationship between oxidative stress, plasma levels of adiponectin and insulin resistance in MONW subjects have not been as yet clarified. In the present study, we investigated the relationship between oxidative stress, circulating levels of adiponectin and insulin resistance in Japanese MONW men.
Material and Methods: We measured the plasma levels of free 8-epi-prostaglandin F2α (8-epi-PGF2α), a marker of oxidative stress, and adiponectin in 28 MONW (age 35.7±1.0 years old, BMI 23.7±0.2 kg/m^2, VFA 130.6± 3.7 cm^2 [mean±SE]) and 23 normal (age 33.4±1.4 years old, BMI 21.0±0.2 kg/m^2, VFA 57.1±4.2 cm^2) men. The plasma levels of free 8-epi-PGF2α were measured using a commercially available enzyme immunoassay (EIA) kit (Cayman Chemical, Ann Arbor, MI) and the plasma levels of adiponectin using a radioimmunoassay kit (LINCO Research, St. Charles, MO). Insulin resistance was evaluated by the euglycemic hyperinsulinemic clamp technique using an artificial pancreas (Nikkiso STG-22, Tokyo, Japan).
Results: The plasma level of 8-epi-PGF2α (30.4±4.0 pg/ml vs. 8.1±1.3 pg/ml, $P<0.01$) in MONW subjects was significantly increased, whereas that of adiponectin (8.6±0.9 μg/ml vs. 11.6±0.7 μg/ml, $P<0.01$) was significantly decreased in MONW subjects as compared to normal subjects. Glucose infusion rate (GIR) during the euglycemic hyperinsulinemic clamp study in MONW men (8.8± 0.5 mg/kg/min) was significantly decreased compared to normal subjects (11.5±0.4 mg/kg/min, $P<0.01$). Significant correlations were observed between VFA and the plasma levels of 8-epi-PGF2α ($r=0.434$, $P<0.05$) and adiponectin

(r=−0.535, $P<0.01$) in MONW men. The plasma levels of 8-epi-PGF2α and adiponectin were significantly correlated in MONW subjects (r=−0.617, $P<0.01$). Both plasma levels of 8-epi-PGF2α and adiponectin were significantly associated with GIR in MONW men (8-epi-PGF2α; r=−0.481, $P<0.01$, adiponectin; r=0.687, $P<0.01$).

Conclusion: The present study showed that systemic increase in oxidative stress and decrease in circulating levels of adiponectin are significantly correlated with insulin resistance in Japanese MONW men. Although correlation does not prove causation, these observations suggest that increased oxidative stress may cause decreased plasma levels of adiponectin and that both abnormalities may contribute to development of insulin resistance in Japanese MONW men.

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The relationship between visfatin levels and anthropometric and metabolic parameters in healthy subjects

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Background and Aims: Adipose tissue has been identified as an endocrine organ. Visfatin is a novel adipocytokine predominantly secreted from visceral adipocytes. Visceral obesity is an important component of metabolic syndrome; however, the relationship between visfatin levels and metabolic syndrome is not clear. The aim of this study was to investigate the association between visfatin levels and parameters of metabolic syndrome and anthropometry in a healthy population.

Materials and Methods: We recruited 198 healthy participants (70 men and 128 women), between the age of 40 to 70 years old. Healthy subjects were defined as individuals with a blood pressure of <140/90 mmHg, normal renal and liver function, a fasting plasma glucose level of <6.1 mmol/L; none of them were taking medication at the time of the study, and all of them were disease free. Anthropometric measurements included height, weight, body mass index, waist and hip circumferences, waist-to-hip ratio and blood pressure. Metabolic parameters including fasting serum visfatin, insulin and plasma glucose, lipid profiles and uric acid levels were measured. Insulin resistance was determined by homeostasis model assessment (HOMA).

Results: Serum visfatin levels in both men and women did not correlate with any markers for adiposity, fasting plasma glucose, fasting serum insulin levels, HOMA or uric acid levels. Visfatin correlated negatively with diastolic pressure in men only after adjusting for age; however, this correlation disappeared after adjusting for age and waist circumference. Visfatin only correlated positively with HDL-C levles in women; this correlation remained unchanged even after adjusting for age. However, this correlation disappeared after adjusting for age and waist circumference. Multiple linear regression analysis using the logarithm of visfatin as a dependent variable revealed that, except for fasting insulin levels and HDL levels in female subjects, non of the anthropometric or metabolic parameters correlated independently with visfatin in either male and female subjects.

Conclusions: Visfatin is not related to anthropometric and most parameters of metabolic syndrome in healthy subjects. The physiologic roles of visfatin in metabolic syndrome warrants further investigation.

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0694

Regulation of white adipose tissue-released factors by insulin and glucose in healthy young men

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Background and Aims: Type 2 diabetes is associated with the dysregulation of white adipose tissue (WAT)-released factors as plasma leptin, ASP, TNFα and TNFα-R1 are elevated while those of adiponectin are decreased in diabetic subjects. However, it is unknown whether this dysregulation is a precursor to, or a consequence of, the diabetic state as studies examining the effect of hyperglycemia (HG) and hyperinsulinemia (HI) on WAT-released factors are scarce in human. Our aim was thus to investigate the role of HG and/or HI in the regulation of WAT-released factors *in vivo* in a healthy subjects.

Population and Methods: We investigated the regulation of plasma concentrations and mRNA expression levels of the following WAT-released factors in response to 3-hour HG and/or HI in healthy lean young men; leptin, adiponectin, ASP (mRNA for which is C3), TNFα, TNFα-

R1 as well as mRNA expression of HKII and p85α PI3K (as positive controls for activation of insulin signaling). Hyperglycemia euinsulinemia clamp (HG, N=8) was induced by constant infusion of 20% dextrose during which endogenous insulin was suppressed by somatostatin and insulin was replaced at baseline levels. Hyperinsulinemia euglycemia clamp (HI, N=10) was induced by a primed-constant exogenous insulin infusion at 75 mU/m^2/min during which glucose was maintained at baseline levels. Hyperglycemia hyperinsulinemia clamp (HGHI, N=7) was a combination of both above clamps. As somatostatin was utilized in both HG clamps, a control somatostatin clamp was also carried out (N=4). All target mRNA expression levels are expressed relative to those of the reference gene HPRT, significance is at $p<0.05$.

Results: Preliminary data indicates that HG clamps (glucose=9.8±1.2 mM, insulin=15.5±4 μU/mL) significantly decreased plasma leptin by 20% (1.8±1.2 *vs* 1.5±1.3 ng/mL) while increased mRNA expression levels of p85α PI3K by 46% (10.2±3.3 *vs* 14.4±4.8). HI clamps (glucose=5.2± 0.4 mM, insulin=138.2±53.9 μU/mL) decreased plasma adiponectin by 15% (11.6±4.8 *vs* 10.0±4.5 μg/mL) while increased plasma leptin by 11% (3.8±2.9 vs 4.2±3.1 ng/mL) and increased mRNA expression of HKII by 147% (20.2± 16.8 *vs* 41.7±23.8) and of p85α PI3K by 150% (20.3±17.2 *vs* 39.1±24.5). HGHI clamps (glucose=10.0±1.2 mM, insulin=154.3±25.6 μU/mL) increased plasma ASP by 21% (30.0±5.7 *vs* 35.6±7.0), and increased mRNA expression of leptin by 130% (8.9±5.5 *vs* 14.0±6.5), of C3 by 27% (10.2±1.9 *vs* 12.8±2.6) and of HKII by 90% (12.9±7.1 *vs* 26.9±13.8). Somatostatin clamp decreased plasma leptin by −17%, which may explain the equivalent suppression in plasma leptin with the HG clamp. There was no significant effect of the HG and/or HI clamps on plasma TNFα or TNFα-R1.

Conclusion: In healthy young lean men, HI suppresses plasma adiponectin and increases plasma leptin while HGHI increases plasma ASP and the WAT-mRNA expression levels of leptin and C3. Thus in diabetes, we hypothesize that elevated plasma leptin and ASP and depressed plasma adiponectin may be a consequence of, rather than a precursor to, HG and HI. On the other hand, the upregulation of the TNFα system that exists in the diabetic subjects may proceed diabetes as HG and/or HI per se had no effect on TNFα or its receptor 1.

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0695

Changes and relations of circulating visfatin, apelin, and resistin levels in normal, impaired glucose tolerance, and type 2 diabetic subjects

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Background and Aims: Visfatin and apelin are two novel adipocyte- secreted hormone proposed to link obesity with insulin resistance. In this study we investigated whether or not plasma visfatin and apelin levels were altered in in normal, impaired glucose tolerance(IGT), and type 2 diabetic(T2DM) subjects. We also assessed the association between plasma visfatin, or apelin and body composition, metabolic parameters, and resistin concentrations in these subjects.

Materials and Methods: Thirty subjects with T2DM, 26 subjects with IGT,37 subjects with obesity, 55 subjects with no obesity and 36 control subjects participated in the study. Anthropometric measurement was performed in the morning, before breakfast, with the subject wearing light clothing, without shoes. Blood samples were drawn at 14 h of fasting and 2 h of glucose overload for determination of plasma glucose, free fatty acid, insulin, resistin, visfatin, apelin and Glycosylated hemoglobin, blood fat levels.Visfatin, apelin as well as resistin were evaluated using a commercially available ELISA. Plasma insulin was measured in deproteinized serum by radioimmunoassay using human insulin as standard. Plasma glucose was assayed using the glucose oxidase method. Glycosylated hemoglobin was measured by isoelectric focusing. Plasma triglyceride and cholesterol concentrations were determined enzymatically. We evaluated for associations between plasma Visfatin, apelin levels and anthropometric (BMI, WHR), metabolic (fasting and overload 2 h glucose and insulin, HbA1c,, FFA, HOMA-IR, lipid profile) parameters, as well as resistin, all expressed as continuous variables.

Results: Fasting and glucose overload 2 h visfatin levels were found to be significantly decreased in the IGT and obesity subjects compared with the controls ($P<0.05$), and visfatin levels were even lower in diabetics than the IGT and obesity subjects ($P<0.05$). In contrast, basal apelin levels were significantly increased in the IGT and diabetic subjects compared with the obesity and controls ($P<0.05$ and $P<0.01$). The apelin levels of glucose overload 2 h were significantly higher than the basal levels in every group (all

$P<0.05$). Fasting plasma visfatin was found to correlate positively and significantly with BMI,WHR, and fasting plasma resistin, but negatively with HbA1c and the blood glucose of overload 2 h. Multiple regression analysis showed that WHR,HbA1c,2hPBG were independent related factors influencing plasma visfatin levels. Fasting plasma apelin levels correlated positively with HOMA-IR,BMI,TC, LDL-C,FBG and Fasting plasma insulin. Multiple regression analysis showed that HOMA-IR,BMI,and TC were independent related factors influencing plasma apelin levels.
Conclusion: These results indicate that visfatin and apelin may play a role in the pathogenesis of insulin resistance and T2DM.
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Adipokines - *in vitro* studies

0696

The novel myokine musclin/osteocrin is induced in human skeletal muscle cells by troglitazone but not by Wy-14,643

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Background and Aims: Recently, a novel myokine named musclin/osteocrin was identified in mice and shown to inhibit insulin action in murine C2C12 myocytes. In this study, we asked whether musclin is expressed in human *in vitro* differentiated skeletal muscle cells (myotubes) as well, and whether agonists of the peroxisome proliferator-activated receptors (PPAR) α and γ modulate musclin mRNA expression.
Materials and Methods: Human myotubes were treated with 5 and 10 μmol/l troglitazone, 5 and 10 μmol/l Wy-14,643, or 0.1% (v/v) dimethylsulfoxide for carrier control. Myocellular mRNA contents were quantified by real-time RT-PCR.
Results: Untreated myotubes expressed 1.51±0.82 fg musclin mRNA/μg total RNA (mean±SD, n=5). These levels were about 3.5-fold higher than that found in a representative biopsy from human skeletal muscle. 20-h treatment of human myotubes with the PPARγ agonist troglitazone dose-dependently increased musclin mRNA expression up to 2.8-fold over control. By contrast, the PPARα agonist Wy-14,643 did not significantly modulate musclin mRNA expression.
Conclusion: In conclusion, we show here that musclin is indeed expressed in human myotubes and that PPARγ activation up-regulates musclin expression.
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0697

The role of visfatin in type 2 diabetes mellitus: the *in vivo* and *in vitro* effects of rosiglitazone, TNFα and IL-6 on visfatin expression in human abdominal subcutaneous adipocytes

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Background and Aims: Visfatin, a novel adipose tissue protein, has been shown to exert insulin mimetic effects in humans and mouse models. However, in hyperinsulinaemic/insulin resistant conditions, visfatin is increased suggesting its' potential role in the pathogenesis of type 2 diabetes mellitus (T2DM). The observed increase may occur directly, via insulin action, or through inducers of insulin resistance, such as TNFα and IL-6, which have been implicated in visfatin regulation. To date, no human studies have explored the role of the insulin sensitiser, rosiglitazone (RSG), TNFα and IL-6 in visfatin regulation. Therefore the aims of these studies were to examine (1) visfatin serum levels in non-diabetic (ND) and T2DM patients; (2) the effect of RSG treatment on circulating visfatin in newly diagnosed T2DM patients; (3) the influence of insulin and RSG on visfatin protein expression in isolated abdominal subcutaneous (AbSc) adipocytes; (4) the role of human recombinant (hr)TNFα and hr IL-6 as regulators of visfatin protein in AbSc adipocytes.
Materials and Methods: Fasting serum samples were obtained from 23 ND and 36 T2DM subjects (ND: BMI: 26.0 (mean±SD)±2.7 kg/m^2; Age: 38.9±12.9 yrs; T2DM: BMI: 35.9±7.2 kg/m^2; Age: 56.0±9.8 yrs) and T2DM patients pre and post 10 week RSG treatment (Age: 57.8±4.0 yrs, BMI: 36.1±2.5 kg/m$^{2;}$, n=8). AbSc and omental (Om) adipose tissue (AT) was obtained from human ND subjects (BMI: 26.5±5.9 kg/m^2, Age 45.7±9.2 yrs) undergoing elective surgery. AbSc adipocytes

were treated for 48 hrs with 100 nM insulin alone or in combination with 10 nM RSG, protein extracted and conditioned media collected. Adipocytes were also treated with hr TNF-α (1–10 ng/mL, n=4) and hr IL-6 (1–10 ng/mL, n=4).

Results: T2DM subjects exhibited higher serum visfatin levels (ND: 13.9(mean±SEM)±1.5 ng/mL vs T2DM: 21.8±3.6 ng/mL, p<0.05) and TNFα levels (TNF-α ND: 11·87±4.3 pg/mL vs T2DM: 16.83±7.0 pg/mL; p=0·007) compared with ND. Post-RSG treated T2DM showed a significant reduction in serum visfatin and insulin compared to pre-RSG treatment (pre-RSG: 10.77±1.47 ng/mL vs post-RSG: 7.45±1.60 ng/mL, p=0.04). RSG 10 nM/insulin 100 nM treated adipocytes showed a significant reduction in visfatin and TNFα protein expression compared with insulin alone (p<0.05), whilst both TNF-α and IL-6 up-regulated visfatin protein expression in these cells (hrTNF-α: Control: 1.0±0.02 (relative fold expression) hr TNFα, 10 nM:1.24±0.06, p<0.05; IL-6: Control: 1.0±0.01, 10 nM: 1.13±0.09, p<0.05). Analysis of visfatin protein expression in human AT showed visfatin was significantly higher in omental AT compared to AbSc AT (p<0.001; n=6).

Conclusion: Circulating visfatin was elevated in T2DM subjects compared to non-diabetic controls. Furthermore, treatment with RSG for 10 weeks lowered circulating visfatin levels in T2DM patients. RSG treated adipocytes reduced visfatin protein expression and TNFα secretion, whilst both hr TNF-α and hr IL-6 increased visfatin expression in adipocytes. Taken together, these data highlight that visfatin is elevated in states where insulin sensitivity is reduced and, as such, visfatin may act as a biomarker for impaired insulin sensitivity.

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0698

Newly identified adipokines secreted by visceral adipose tissue and their deregulation in obesity

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Background and Aims: Obesity, and more particularly central obesity, is strongly associated with chronic disorders such as type 2 diabetes, cardiovascular disease and cancer. Adipokines are involved in the pathogenesis of these obesity-linked disorders. However, adipokines secreted by visceral adipose tissue (AT), unlike those secreted by other fat depots, are still poorly characterised. In this study, we searched for novel adipokines secreted by visceral AT, compared their production in lean versus obese subjects and examined the relative contribution of the different cell types to this production.

Subjects and Methods: Visceral AT was obtained from 7 obese (age, 46±7 yr; body mass index (BMI), 42±3 kg/m^2) and 7 non-obese (age, 51±7 yr; BMI, 24±1 kg/m^2) men undergoing elective abdominal surgery. AT was digested by collagenase treatment into isolated adipocytes (A) and stromal-vascular cells (SVC). These cellular fractions were cultured separately for 24 h. In another experiment, cultured preadipocytes were differentiated over 12 days into mature adipocytes.

Results: We screened culture medium of A and SVC using a commercial array allowing the detection of 120 cytokines. We identified 16 cytokines secreted by each cellular fraction of visceral AT in lean and obese subjects. Out of them, 6 adipokines were newly identified as secretory products of visceral AT. These adipokines include 3 chemokines, 1 interleukin, 1 tissue inhibitor of matrix metalloproteinases and 1 hematopoietic growth factor. A and SVC secreted rather similar amounts of these adipokines, when data were normalised on a cellular basis. However, for each fraction, this secretion was enhanced in obese subjects compared to lean ones (from 2- up to 8- fold, P<0.05). Qualitatively similar results were obtained when mRNA levels of these adipokines were analysed by real-time RTQ-PCR. The mRNA concentrations of 5 of these 6 adipokines markedly increased during *in vitro* adipogenesis (6- to 18- fold, P<0.05 or less versus preadipocyte state).

Conclusion: Six adipokines were newly identified in visceral AT. The expression and secretion of these adipokines were increased in obesity, with a relatively similar contribution of A and SVC to this enhanced production. Because of their already known involvement in cardiovascular disease, cell proliferation or insulin resistance, these adipokines may play a role in obesity-linked adverse health outcomes.

0699

Protein array reveals changed levels of several cytokines in adipose tissue of obese subjects with features of metabolic syndrome

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Background and Aims: The role of adipokines and inflammatory cytokines of adipose tissue for development of obesity-related insulin resistance has not yet been completely understood. Therefore, the aim of the study was 1) screened the protein level of 120 cytokines in subcutaneous adipose tissue (ScAT) of lean and obese subjects and 2) compared the gene expression for selected cytokines in ScAT in basal state and after euglycemic hyperinsulinemia (EHI).
Materials and Methods: All recruited subjects initially underwent an oGTT, and those with IFG, IGT and DM2 were excluded. Thus, 18 drug naive Caucasian males (12 obese (OB): BMI 33.5±0.9 kg/m^2, age 26.8±2 yrs and 6 lean control (C): BMI 21.5±0.5, age 28.7±1) were included into the study. OB had increased fasting plasma glucose, insulin, hsCRP, Tg and HDL-Ch levels, decreased *in vivo* insulin action (assessed by euglycemic hyperinsulinemic clamp [EHC]) ($p<0.01$). ScAT biopsies were performed after an overnight fast and in the 3rd hour of EHC. Protein levels in basal state were assessed in tissue lysates using the RayBio®Human Cytokine Antibody Array C Series 1000.
Results: From 120 cytokines measured, 27 presented higher (leptin, HGF, EGF-R, FGF-6, IGF-1sR, Fas, ENA-78, PARC, lymphotactin, HCC-4, IL-10, IL-1a, IL-1R1, IL-1R4, IL-12p70, angiopoietin-2, Axl, Dtk, MIF, MIP-1a, -1b, -3b, MSP-a, OPG, TECK, TIMP-1, −2) and 1 (RANTES) presented lower levels in ScAT of OB subjects in comparison with C ($p<0.05$). Furthemore in the OB, leptin had increased also ScAT mRNA (real-time RT-PCR) and plasma levels; TNFα had increased mRNA only, and IL-6 and adiponectin did not differ at any level studied. EHI led to further elevation of the mRNA levels for leptin and IL-6 in OB and C ($p<0.05$).
Conclusion: Our data provide new evidence on a very early dysregulation of cytokine ScAT levels in clinically yet healthy OB subjects. Further amplification of the changes by EHI may indicate toward a possible perturbation of the situation in response to endogenous hyperinsulinemia in obesity.
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0700

Comparison of super-coiled minicircle with conventional plasmid in systemic non-viral adiponectin gene therapy to ameliorate insulin resistance in diet induced obese mice

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Background and Aims: Gene therapy is an attractive tool to be used for the treatment of various human diseases. However, several serious harmful side effects of widely used viral gene carrier limit its clinical usages. Non-viral gene delivery might be an alternative to avoid the immune response and the illegitimate DNA integration to the host chromosome. Despite of the superior biological safety, non-viral gene delivery has suffered from its weak transfection efficiency as compared with the viral gene therapy. Adiponectin is a very famous adipocytokine that can improve many biological markers of insulin resistance. But its blood concentration is so high to be increased by conventional non-viral gene therapy technique. To increase the transfection efficiency of non-viral gene delivery, we performed this study.
Materials and Methods: We cloned small sized novel super-coiled minicircle plasmid pMINI-beta-ADN(adiponectin) devoid of bacterially originated CpG motifs. We injected this plasmid complexed with the cationic polymeric carrier into the tail veins of DIO(diet induced obese) C57BL/6J mice.
Results: Tail vein injection of pMINI-beta-ADN showed 1.5–2.0 fold increase of blood adiponectin level. Blood glucose, free fatty acid and HOMA-IR values were normalized. RT-PCR revealed that the adiponectin gene expression mainly took place at the liver and spleen, and the main oligomer form of the increased adiponectin in blood was HMW(high molecular weight) form which was verified by Western blot analysis. On the contrary, the injection of conventional plasmid pBETA-ADN did not increase the blood adiponectin level.
Conclusion: These data might suggest that the non-viral gene therapy using minicircle plasmid could be successfully employed to increase the blood level of therapeutic peptides including adiponectin, one of the most abundant proteins in the human blood.

0701

Elevated expression of visfatin in streptozocin-induced diabetic rats

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Background and Aims: Visfatin, also known as pre-B cell colony-enhancing factor (PBEF), was identified as a novel adipocytokine exerting putative antidiabetogenic properties. Previous studies have shown that PBEF is not only expressed in many important tissues, but the protein lacks a signal sequence, so it is necessary to clarify the origin of serum visfatin. This study was to investigate the relationship between visfatin serum level and its expression in tissues in a model of streptozotocin (STZ)-induced diabetic rats.
Materials and Methods: 8 week old male Sprague-Dawley (SD) rats were chosen. Hyperglycaemia was induced by intravenous STZ injection at 65 mg/kg bodyweight (n=12). citrate buffer-injected rats were chosen as parallel control (n=12). Blood glucose and bodyweight were monitored. Rats were sacrificed at indicated time, tissues (heart, epididymal adipose tissue, skeletal muscle, liver, thoracic aorta) and serum were harvested. Expression of visfatin in various tissues was investigated by Northern blotting, and serum protein level was measured by immunoblotting.
Results: Diabetic rats remained hyperglycemic (10.8± 3.4 mmol/L at 4th week and 19.7±4.1 mmol/L at 12th week; n=6, respectively, P<0.01 vs control) after confirmation of hyperglycemia (>10 mmol/L). Visfatin mRNA expression was observed in all the tissues tested, with the maximum in liver and the least in thoracic aorta. Its expression was significantly high in all tissues of diabetic rats as compared to parallel control ones (P<0.05), and a remarkable increase was observed in liver and epididymal adipose tissue of diabetic rats (3- and 4-fold, respectively; P<0.01 vs control). Serum visfatin protein level mirrored its mRNA expression in tissues both in diabetic rats and normal control ones (r=0.31, P<0.05). Furthermore, diabetic course seemed have no influence on visfatin expression in various tissues and serum level as well.
Conclusion: Visfatin is expressed universally and constantly *in vivo* with a highest level in liver under physical condition. Its Increased circulating level in parallel with upregulated expression in tissues under diabetic milieu probably indicates that visfatin, through its insulin-mimetic metabolic effects, might play a compensative role in the course of diabetes and total body tissues, besides adipose, may contribute to the change in serum visfatin level.
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0702

Pioglitazone effect on plasma, visceral and subcutaneous adipose tissue adiponectin of fatty Zucker rats

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Background and Aims: Adiponectin is an insulin-sensitizing hormone with anti-inflammatory properties which is reduced in insulin resistance states like obesity and type 2 diabetes.
Thiazolidindiones are PPAR-γ agonists that improve insulin resistance and upregulate adiponectin levels in plasma and adipose tissue. It has been proposed that adiponectin upregulation could be the mechanism used by thiazolidinediones to improve insulin resistance linked to obesity.
The aim of this study is to know if the amelioration of insulin resistance produced by pioglitazone is associated with changes in the adiponectin content in plasma, visceral and subcutaneous adipose tissue in Zucker fatty rats, an animal model that associates extreme obesity, insulin resistance and dysregulation of lipidic metabolism.
Materials and Methods: Fatty Zucker rats were treated with pioglitazone (3 mg/kg/d, n=10) or placebo (n=10) for 12 weeks. Blood was collected weekly, and at the end of the treatment rats were sacrificed by intraperitoneal injection of sodium penthotal, visceral and subcutaneous adipose tissue, and blood were collected.
Measurements: Plasma insulin by RIA, plasma cholesterol and triglycerides by enzymatic assays, plasma and visceral and subcutaneous adipose tissue adiponectin content by Western-blot.
Results: Compared to placebo, rats treated with pioglitazone had a significant decrease (p<0.05) of plasma insulin levels (16.8±2.2 vs 3.4±0.4 ng/ml), plasma cholesterol (147.1±5.0 vs 110.7±5.7 mg/dl) and plasma triglycerides (235±14.1 vs 141.7±9.3 mg/dl). Pioglitazone treatment, compared to placebo, produced a significant increase (p<0.05) in plasma adiponectin levels (1.34±0.12 vs 0.60±0.10 AU), and in adiponectin protein content of visceral adipose tissue (1.12±0.13 vs 0.51±0.06 AU) and subcutaneous adipose tissue (1.54±0.28 vs 0.79± 0.08 AU).
Placebo treated rats presented lower adiponectin protein content in visceral adipose tissue compared to subcutaneous adipose tissue (p<0.05), whereas pioglitazone treated rats had similar adiponectin protein content in both tissues.

Plasma adiponectin correlated negatively ($p<0.05$) with HOMA value ($R=-0.71$), cholesterol ($R=-0.58$) and triglycerides ($R=-0.71$).

Conclusion: Pioglitazone produces an increase of adiponectin in plasma and adipose tissue that is associated with amelioration of insulin sensitivity and lipidic metabolism.

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0703

Acute and chronic treatments with a new recombinant globular domain of adiponectin: improvement of insulin sensitivity, glucose tolerance and FFA metabolism in the high fat diet-fed diabetic mouse

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Background and Aims: Adiponectin increases glucose transport, reduces inflammation, and controls vascular function. Hence, we propose that treatments with a recombinant globular domain of adiponectin (aa 110–244), designated AS100-244, exhibit significant therapeutic potential for hyperglycemic syndromes.

Materials and Methods and Results: Mice were fed for three months a high fat-carbohydrate free diet (HFD) to induce diabetes and mild obesity. In the first set of experiments, the mice were infused intravenously with different doses of AS100-244 (0.12; 0.4 and 1.2 μg/kg/min) for 5 hours. Basal and insulin sensitive glucose utilization rates were assessed by the use of a continuous tritated glucose infusion and pharmacological dose of insulin (18 mU/kg/min) in the awake free moving mouse. The data showed that AS100-244 dose dependently reduces insulin resistance by increasing whole body glucose utilization (35% at the highest dose) and glycolysis rate of the HFD mice. Similarly, the reduction of FFA concentration by insulin was dramatically improved and fastened. However, in the basal fasting state hepatic glucose production was unchanged by AS100-244 infusion. This acute improved glucose homeostasis was associated with an increased content of muscle phospho-Akt and AMP-activated kinase. In a second set, HFD mice were chronically treated with a twice daily s.c AS100-244 injection at three doses (10, 30 and 100 μg/kg). Fasted glycemia, glucose tolerance as well as insulin sensitive glucose utilization were improved by AS100-244 at the highest dose after 3 weeks of treatment with concomitant decrease in body weight gain when compared to vehicle treated mice. The metabolic improvements were similar to those obtained by a metformin treatment.

Conclusion: Our data show that AS100-244, a recombinant globular domain of adiponectin, represents an efficient therapeutic strategy for the treatment of hyperglycemic and insulin resistant syndromes.

0704

Serum adiponectin levels in relation to the tissue insulin resistance in obese and non-obese models of metabolic syndrome

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Background and Aims: Adiponectin is adipocytokines with antidiabetic properties. Its plasma concentration is decreased in obesity and type 2 diabetes. Adiponectin is believed to positively influence insulin sensitivity but information concerning regulation of its secretion in insulin resistance which is not associated with obesity is still missing. We investigated the relationship between serum concentration of adiponectin and visceral adiposity, triglyceridemia and the peripheral tissue insulin sensitivity in obese and non-obese experimental models of insulin resistance. Further the effect of high fructose diet on adiponectin levels was examined.

Materials and Methods: The experiments were carried on adult male a) non-obese hereditary hypertriglyceridemic rats (HHTg) 4- and 18- month old, b) obese Zucker rats and c) non-obese spontaneously hypertensive rats (SHR) fed a high carbohydrate diet (60% of fructose) for two weeks. Age- and strain-matched non-obese rats served as controls. The adiponectin was determined by ELISA kit (B-Bridge Inc., USA). The resistance of muscles and adipose tissue to insulin was estimated according to the insulin stimulated ^{14}C-U-glucose incorporation into glycogen and lipids.

Results: In obese rats, the low adiponectin levels in serum were associated with higher epididymal fat pad weight, with significant hypertriglyceridemia a nearly complete resistance of adipose and skeletal tissue to the insulin stimulated glucose utilization for lipid and glycogen synthesis. In contrast, in HHTg and control rats the serum adiponectin concentration were not different at the age 4 and 18 months in spite of higher triglyceridemia and impaired insulin sensitivity of skeletal and adipose tissue in HHTg rats. The age-related drop in adiponectin serum concentrations by 30% was similar in 18-month-old HHTg and control rats although icreased hypertriglyceridemia and muscle and adipose tissue insulin resistance in HHTg rats. High fructose feeding increased triglyceridemia and periph-

eral insulin resistance in HHTg rats, and against expectation serum adiponectin levels were increased both in 4-mo old HHTg rats by 96% and in 18-mo old rats by 190%. This diet increased serum adiponectin similarly in 4-mo old control rats by 66% and in 18-mo old rats by 190%. Fructose-induced hypertriglyceridemia and insulin resistance in SHR rats had no effect on serum adiponectin concentration.

Conclusion: Our results suggest contribution of down-regulation of adiponectin secretion to the development of age-dependent deterioration of insulin sensitivity. The correlation between epididymal fat pad mass and serum adiponectin concentration ($r^2=-0,82$; $p<0,02$) supports the hypothesis about the relationship between adiponectin secretion and the adipose tissue mass. In contrast to this neither inherited hypertriglyceridemia nor nutritionally induced insulin resistance were not associated with different adiponectin serum concentration.

Group	Epididymal fat pads, g/10	Serum TG mmol/l	Serum adiponec,μg/ml
Zucker obese, 4-mo old	2.75±0.09	2.81±0.26	1.86±0.17
K-Wistar, 4-mo old	1.55±0.07	0.85±0.07 [a]	3.17±0.31 [a]
K-Wistar, 18- mo old	1.72±0.07	1.17±0.07 [a]	2.25±0.12 [a]
HHTg, 4-mo old	1.39±0.06 [b]	2.95±0.21 [b]	3.32±0.18 [b]
HHTg, 18-mo old	1.87±0.09 [b]	3.83±0.41 [b]	2.44±0.20 [b]
SHR, 4-mo old	1.05±0.05	0.54±0.09 [c]	6.16±0.61
SHR 4-mo old+ fructose	1.05±0.04	0.99±0.18 [c]	5.63±0.92

The same symbols represent statistical significance $p<0.05$.

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PS 52

Adipose tissue metabolism

0705

Modulation of lipolysis, lipogenesis and glucose uptake through human adipocyte differentiation: evaluation of insulin sensitivity

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Background and Aims: Currently there is strong evidence that dysfunction of adipose tissue plays a crucial role in the development of insulin resistance associated to Obesity and Type 2 diabetes. Adipose tissue not only responds to afferent signal from traditional hormone systems but also expresses and secretes factors with important endocrine functions, a fact that is emphasized by the adverse metabolic consequences of fat excess. Human adipocyte research is hampered by the lack of human fat cell lines. So most studies are performed on immortalized rodent cell lines such as 3T3-L1, murine cells with several metabolic and signal transduction differences compared with human cells; or primary cultures of human preadipocytes, which are obtained from fibroblast-like cells isolated form the stromal portion of human fat tissue. These cells cannot be expanded and display only a limited life span. Moreover, the reduced availability of human adipose tissue is a limitation for more detailed studies. Therefore, the purpose of the present study was to characterize a novel human adipocyte cell line that expresses differentiated functions, particularly referred to glucose and lipid metabolism.

Materials and Methods: LiSa-2 is a stable cell line derived from a poorly differentiated human liposarcoma. In serum-containing medium they are fibroblastoid and rapidly dividing but when grown in adipogenic medium, LiSa-2 cells differentiate into adipocyte-like cells with several phenotypic, biochemical and functional characteristics of mature human fat cells. In addition to the protein expression pattern, and insulin signaling pathway, representative metabolic functions of adipose tissue such as glucose uptake, lipogenesis and lipolysis were investigated. Studies were performed on undifferentiated (0 days of culture) and confluent differentiated cells (7, 14 and 21 days of culture). Moreover, the effect of cytokines (TNF-α and IL6) and agonists of different nuclear receptors (PPARγ, LRX, RAR, and RXR) on glucose uptake and lipolysis were also analyzed.

Results: Differentiation of LiSa-2 cells was associated with expression of several adipocyte markers and sensitivity to lipolysis and glucose uptake induced by insulin, reaching maxim levels at 14 days of differentiation. However, prolonged cell cultures gradually lose the expression of some proteins such as GLUT4 glucose transporter. These changes in gene expression contribute to the development of insulin resistance in LiSa-2 cells and mimic the insulin-resistant phenotype in adipose tissue *in vivo*. On the other hand, in differentiated and insulin sensitive cells TNF-α but not IL6 induce lipolysis and caused insulin resistance. Treatment with different nuclear receptor agonists differentially regulate glucose and lipid metabolism under physiological and insulin-resistance conditions.

Conclusion: LiSa-2 is a novel human adipocyte cell line that may serve as a well-established *in vitro* cell model as an alternative to primary cultures. Studies with this human cell line is likely to reveal further insight into the endocrine

function of adipose tissue and the molecular mechanisms involved in insulin resistance states associated to Obesity. This knowledge may permit more rational approaches to treatment of the metabolic consequences of excess of adipose tissue.
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0706

WISP-2, a Wnt-signaling protein, is increased in obesity and adipocyte enlargement

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Background and Aims: In recent years the importance of the adipose tissue for the development of type 2 diabetes and its complications has become evident. type 2 diabetes and insulin resistance has been shown to correlate with different markers of adipocyte differentiation and, thus, function. The Wnt-signaling pathway is a well known regulator of cell cycle progression and an inhibitor of cell differentiation. Recent studies in our laboratory have shown that exposure of 3T3-L1 preadipocytes to cytokines results in maintenance of Wnt-signaling and inhibition of adipocyte differentiation. In this study, we have focused on the gene expression of WISP (Wnt-1 inducible signaling protein) −2, a Wnt-signaling responsive gene, and its relation to adipocyte differentiation and risk factors for type 2 diabetes, such as obesity and circulating IL-6 levels.
Material and Methods: To study the expression of WISP-2 in human adipocytes, we obtained abdominal subcutaneous adipose tissue by surgical incision from 10 type 2 diabetic subjects before and after treatment with the insulin sensitizer, pioglitazone. To study WISP-2 in pre-diabetic patients, we also obtained adipose tissue biopsies from 36 well-characterized first-degree relatives to type 2 diabetic patients. Furthermore, we have cultured and differentiated human preadipocytes from 6 donors to study WISP-2 expression during adipogenesis.
Results: The expression of WISP-2 in adipose tissue from type 2 diabetic patients correlated with several risk factors for type 2 diabetes such as BMI (r=0.85, p=0.002) WHR (r=0.71, p=0.02) and circulating IL-6 levels (r=0.61, p=0.06)but not to insulin sensitivity. The expression of WISP-2 was reduced by 20% after pioglitazone treatment (p=0.06), but the reduction did not correlate with the improvement of the insulin resistance. In the pre-diabetic group, WISP-2 expression in adipose tissue was also positively correlated to waist (r=0.34, p=0.04), WHR (r=0.30, p=0.07) and cell size (r=0.42, p=0.01), but not to insulin sensitivity. In the human preadipocytes, we found that the expression of WISP-2 was significantly reduced during adipocyte differentiation, while the presence of IL-6 counteracted this decrease. This is in agreement with the positive correlation between circulating IL-6 levels and the WISP-2 expression in adipose tissue seen in the diabetic group.
Conclusion: Taken together, these data suggest that Wnt-signaling is increased in adipocytes from obese subjects and subjects with enlarged adipocytes. Furthermore, the expression of the wnt-signaling responsive gene WISP-2 correlates with known risk factors for type 2 diabetes.
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0707

Mesenchymal stem cell differentiation into adipocytes is equally induced by insulin and proinsulin *in vitro*

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Background and Aims: In advanced β-cell dysfunction stage III, proinsulin (PI) is increasingly replacing insulin (INS) as major component of the secretion product. Proinsulin has only 10–20% of the glucose lowering activity of INS, but it has been speculated that PI has at least the same adipogenetic potency than INS, leading to an increased tendency of lipid tissue formation in patients with late stage β-cell dysfunction. Since the hormonal secretion of the growing adipose tissue is a driver for insulin resistance, the increased PI amounts required to lower glucose may be strongly promoting the further deterioration of the metabolic situation. The purpose of this experiment was to investigate the adipogenetic effects of PI in comparison to that of INS.
Materials and Methods: Mesenchymal stem cells obtained from liposuction material were grown in differentiation media containing INS (0.01 μMol), PI (0.01 μMol) or PI +INS (each 0.005 μMol). Cell culture supernatants were taken from these experiments and an untreated control at weeks 1, 2, and 3, and were stored at − 80°C until analysis. Cell differentiation was microscopically supervised and confirmed by Oilred-O staining, and adiponectin concentrations

were measured as marker for differentiation into mature lipid cells. This experiment was repeated three times.
Results: No growth of lipid cells and no change in adiponectin values was observed in the negative control group (3.2±0.5/3.3±0.1/4.4±0.5 ng/ml). A continuous differentiation into mature adipocytes (also confirmed by Oilred-O-staining) and a corresponding increase in adiponectin values was observed in the experiments with INS (3.6±1.9/5.1±1.4/13,3±1.5 ng/ml; $p<0.05$ week 1 vs. week 3) and PI (3.3±1.2/3.5±0.3/12.2±1.2 ng/ml; $p<0.05$). Comparable effects were also seen with the INS+PI combination (3.5±0.7/4.0±0.2/14.6±3.0 ng/ml; $p<0.05$).
Conclusion: Proinsulin has the same adipogenetic potential than insulin *in vitro*. Since the blood glucose lowering potency of PI is much lower than that of INS, much more PI needs to be secreted to control blood glucose *in vivo*. It can be speculated that the adipogenetic potential of proinsulin may be a large contributor to the increased body weight problems in patients with type 2 diabetes and advanced β-cell dysfunction.

0708

High subcutaneous interstitial levels and trivial spillover into the circulation implicate monocyte chemoattractant protein-1 as a paracrine regulator

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Background and Aims: The chemokine monocyte chemoattractant protein-1 (MCP-1), known to be produced by the adipose tissue (AT), has been strongly implicated in the obesity-associated macrophage infiltration into AT, insulin-resistance, and inflammation. The aims of the present study were: 1) to evaluate the contribution of adipose-derived MCP-1 to the systemic levels by combining the *in situ* calibrated microdialysis technique with serum measurements; 2) to characterize the gene expression pattern in AT before and after a euglycemic hyperinsulinemic glucose clamp.
Material and Methods: Nine lean (L) (Age: 34.6±3.4 yr; BMI: 22.5±0.8 kg/m^2) and 9 uncomplicated obese (OB) (Age: 32.8±2.6 yr; BMI: 34.7±1 kg/m^2), male subjects were studied in the fasting state, and during a 4-hr euglycemic hyperinsulinemic glucose clamp (40 mU/m^2/min). Moreover, serum (s) and interstitial (i) MCP-1 measurements were estimated. A needle biopsy from the abdominal subcutaneous AT was taken at baseline and after 4-hr of insulin infusion to examine the gene expression profiles.
Results: OB showed slightly higher basal sMCP-1, as compared with L (352±38 *vs* 271±22 pg/ml, $p=0.07$). Basal iMCP-1 was ~4- and ~7-fold higher than sMCP-1 ($p<0.001$) in OB and L, respectively. During the clamp no changes of sMCP-1 occurred. Among the OB, basal AT gene expression pattern revealed an up-regulation of MCP-1 ($p<0.01$) and CD68 ($p=0.02$), coupled with a down-regulation of GLUT-4. Insulin infusion increased MCP-1 and GLUT-4 in the L ($p<0.05$) but not in OB, showing a differential regulation by insulin in these groups.
Conclusion: These observations show: 1) high subcutaneous interstitial MCP-1 levels and trivial spillover into the bloodstream implicating adipose-derived MCP-1 as a paracrine/autocrine modulator; 2) acute hyperinsulinemia does not affect circulating MCP-1 concentrations, but increases GLUT-4 and MCP-1 gene expression in the adipose tissue in lean but not in obese subjects.
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0709

Systemic activation of the endocannabinoid system in adipose tissue of obese and type 2 diabetic patients

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Background and Aims: Obesity is one of the main risk factors for type 2 diabetes mellitus (T2DM). Activation of the endocannabinoid system (ECS) promotes appetite and weight gain. This process can be inhibited by blocking of the cannabinoid receptor 1 (Cb1), which is expressed in neuronal and immune cells as well as in adipose tissue (subcutaneous and visceral). In obesity activation of the ECS was demonstrated by elevated serum levels of endocannabinoids and reduced expression of Cb1 and fatty acid amide hydrolase (FAAH) in subcutaneous adipose tissue. FAAH plays a significant role in degradation of circulating endocannabinoids and is therefore known as a negative regulator of the ECS. So far it is unknown if the expression of Cb1 and FAAH is only reduced in subcutaneous adipose tissue or if the reduction also is present in visceral adipose tissue and if metabolic dysregulation like T2DM affects their expression.
Materials and Methods: Using quantitative RT-PCR we analyzed the expression of Cb1 and FAAH in visceral and

subcutaneous adipose tissue of non-diabetic subjects with normal (visceral: n=10, BMI 24.4±1.1; subcutaneous: n=18, BMI 23.8±1.9) and with strongly elevated BMI (visceral and subcutaneous: n=11, BMI 37.6±13.6) as well as in subcutaneous adipose tissue of T2DM patients (n=5, BMI 25.0±2.0 and n=8, BMI 35.0±5.2).

Results: In non-diabetic obese subjects the expression of Cb1 (30%) and FAAH (60%) was reduced in visceral adipose tissue compared to normal weight controls. Moreover, we could show that in comparison to non-diabetic subjects the expression of Cb1 (70%) and FAAH (45%) was strongly reduced in subcutaneous adipose tissue of T2DM patients, with further reduction of mean Cb1 expression by obesity in the non-diabetic (80%) as well as in the diabetic (50%) group.

Conclusion: Our results suggest that obesity as well as T2DM independently reduce the expression of Cb1 and FAAH, which might be explained by systemic activation of the ECS in adipose tissue.

0710

Lipin (Lpin1) expression is decreased in subcutaneous adipose tissue in human obesity

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Background and Aims: Lpin1 gene is emerging as an important determinant of adipose tissue differentiation and has been related to lipodystrophy and to obesity in animal models. The purposes of this analysis were to compare lipin adipose tissue gene expression levels in obese subjects and patients with type 2 diabetes and to assess if its expression parallels to animal models in human adipocyte differentiation.

Materials and Methods: Subcutaneous adipose tissue samples were obtained from 44 healthy lean and obese subjects and from 48 patients with type 2 diabetes matched for body mass index (BMI), whose clinical and anthropometrical variables were recruited. Real Time PCR was used to measure gene expression of Lpin1, PPARγ and SREBP1c in whole adipose tissue and of Lpin1 in *in vitro* differentiated subcutaneous- and visceral-isolated preadipocytes.

Results:In non-diabetic subjects, Lpin1 mRNA expression levels were significantly reduced in the obese group (7.57±0.11 vs 7.23±0.07; p=0.013), but no differences with type 2 diabetes patients were observed. In the multiple regression analysis, after adjusting for confounding variables only BMI was negatively associated with Lpin1 expression (β=−0.354, p=0.01). Lpin1 mRNA expression increased with preadipocyte differentiation but the increment was higher in subcutaneous than in visceral adipose tissue.

Conclusion: Human obese subjects express lower Lpin1 mRNA levels than lean subjects in subcutaneous adipose tissue and its expression is not altered by the presence of type 2 diabetes mellitus. Lpin1 expression is induced during human adipocyte differentiation *in vitro*.

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0711

WITHDRAWN

0712

Preadipocyte factor 1 (Pref-1/DLK1/FA1) in obesity and insulin resistance

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Background and Aims: Preadipocyte differentiation occurs during distinct periods of human development and is a key determinant of body mass. Preadipocyte factor 1/ Delta like factor 1 (Pref-1/DLK-1) is a gene encoding a transmembrane protein that undergoes proteolitic cleavage to generate a soluble form called in humans, Fetal antigen 1 (FA1). Pref-1/DLK-1 constitutive expression *in vitro* led to inhibition of osteoblast and adipocyte differentiation on human stromal cells an on 3T3L-1 mouse cells inhibits its differentiation to adipocyte.

Our aim was to investigate if there was variation in committed preadipocytes between individuals with different degree of obesity. We also aimed to find any correlation between circulating levels (FA1) and its local adipose tissue expression and insulin resistance.

Materials and Methods: FA1 were measured by ELISA technique in n=134 consecutive healthy male subjects to

whom insulin sensitivity (S_i) test was performed. Circulating levels of sCD14, sTNFR2, sIL-6 and adiponectin were also measured in this population.
Subcutaneous adipose tissue samples were obtained from a different cohort in non-diabetic (n=40) and patients with Type 2 diabetes (n=16) with different degrees of obesity. Real Time PCR was used to measure gene expression of *pref-1*. FA1 plasma levels were also measured in this group.
Results: In healthy male cohort, after multiple regression analysis, S_i ($\beta=-0.180$, $p<0.05$) and HDLCholesterol ($\beta=-0.191$, $p<0.039$) appeared as independent determinants of FA1 plasma levels.
Pref-1 mRNA expression levels in subcutaneous adipose tissue did not show differences between obesity groups or with the presence of Type 2 diabetes. In this cohort, FA1 levels correlate positively with age, waist and hip ratio (WHR), soluble fraction of tumor necrosis alpha receptors TNFR1 and TNFR2 (r=0.287, p=0.009; r=0.276, p=0.027; r=0.356, p=0.004 and r=0.354, p=0.003, respectively). After controlling for confounding variables, only WHR ($\beta=0.375$, p=0.002) and sTNFR2 ($\beta=0.352$, p=0.004) remained as the independent determinants of FA1 levels.
Conclusions: Increased FA1 circulating levels may be representatives of an increased cardiovascular risk, including low insulin sensitivity. Adipose tissue expression does not contribute in a relevant manner to FA1 plasma concentration.
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0713

Increased glucose tolerance despite low adiponectin levels in obesity-resistant aP2-*Ucp1* transgenic mice fed a high-fat diet

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Background and Aims: Transgenic mice with ectopic expression of mitochondrial uncoupling protein 1 (UCP1) in white fat (aP2-*Ucp1* mice) show partial resistance to obesity induced by a high-fat (HF) diet, which is associated with a preferential reduction of subcutaneous fat depots, low plasma triacylglycerols, and reduced lipid content in the muscle and liver. Despite these beneficial effects, transgenic mice are hyperglycemic in the fasted state, especially when fed a standard chow. In order to understand the mechanims involved in the control of glucose metabolism in the aP2-*Ucp1* mice, we analyzed glucose homeostasis, plasma concentrations of adipokines, as well as insulin sensitivity in the aP2-*Ucp1* transgenic mice and their C57BL/6J (B6) littermate controls.
Materials and Methods: Male transgenic aP2-*Ucp1* and their B6 littermate controls were fed a standard chow until 3 mo of age, when some mice were assigned to a HF diet (60% calories as fat). Basal hepatic glucose production (HGP) was measured by continuous D-(3-^{3}H)glucose infusion into fasted mice fed a standard chow. Glucose turnover (GTO) was assessed by hyperinsulinemic-euglycemic clamps (a primed continuous infusion of human insulin ~4 mU/kg.min, blood glucose clamped at 5.5 mM) in overnight fasted mice after a short-term (3 wks) HF feeding. Glycemic control in mice fed a HF diet for 2 mo was assessed by intraperitoneal glucose tolerance test and expressed as area under the curve (AUC). Plasma levels of adiponectin and leptin were measured by ELISA, while gene expression in the skeletal muscle by quantitative real-time RT-PCR.
Results: Fasted glycemia (~ 15 hr fast) in the chow-fed transgenic mice was in a range of 109 to 135 mg/dL, while in B6 controls the range was 87–103 mg/dL. However, basal HGP was only slightly elevated in the transgenic mice compared to controls (15.1±1.3 vs. 12.8±0.8 mg/kg.min), while GTO was not different between the genotypes (aP2-*Ucp1*, 60±3 vs. B6, 70±9 mg/kg.min). Plasma levels of adiponectin, a negative regulator of HGP, were decreased by ~ 30% in the aP2-*Ucp1* mice. Short-term HF feeding significantly lowered GTO in both strains to the same extent (aP2-*Ucp1*, 48.3±3.8 vs. B6, 51.5±6.0 mg/kg.min). Despite the absence of detectable differences in insulin sensitivity after a short-term HF diet, glucose tolerance was significantly higher in the transgenics compared to B6 controls after 2 mo of HF feeding (AUC: aP2-*Ucp1*, 1105±97 vs. B6, 1625±127 mM.min). Better glycemic control of transgenic mice compared to B6 controls was associated with a higher expression of *Glut4* in the skeletal muscle (by ~ 44%) despite lower plasma levels of adiponectin (aP2-*Ucp1*, 4.5±0.4 vs. B6, 8.2±0.4 μg/mL) and leptin (3.3±0.5 vs. 6.2±1.7 ng/mL).
Conclusion: Respiratory uncoupling in white fat of the aP2-*Ucp1* transgenic mice was associated with low adiponectin levels that could be responsible for elevated HGP in these mice. UCP1 over-expression in white fat did not affect the impairment of whole-body insulin sensitivity induced by a short-term HF feeding, however it partially protected against glucose intolerance and down-regulation of *Glut4* in skeletal muscle induced by a prolonged HF feeding, likely due to its ability to protect non-adipose tissues against the accumulation of lipids.

0714

Significance of white adipose tissue metabolism for whole body nutrition partitioning

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Background and Aims: Studies on various transgenic mice indicated that accumulation of body fat can be decreased by specific modulation of metabolism of white adipose tissue (WAT), in spite of the fact that WAT contributes marginally to whole body energy expenditure. We used the model of mice resistant to obesity due to ectopic expression of mitochondrial uncoupling protein 1 (UCP1) in WAT (aP2-*Ucp1* mice) to study the effect of respiratory uncoupling in WAT mitochondria on whole body energy expenditure and nutrition partitioning. The effect of n-3 polyunsaturated fatty acids of marine origin, eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, which limit accretion of body fat while up-regulating mitochondrial biogenesis and increasing fatty acid oxidation in WAT was also studied.
Materials and Methods: To characterize the effect of ectopic UCP1 in WAT, aged male hemizygous aP2-*Ucp1* mice and their nontransgenic littermates on C57BL/6J background fed chow diet *ad libitum* and maintained at 30°C were used. Body weight was lower in transgenic than nontransgenic mice (34.1±0.7 vs. 38.1±0.9 g; n=8). To study the effect of EPA and DHA, adult male C57BL/6J were maintained at 20°C and fed for 2 wk either high-fat (HF) diet (60% calories as fat, mainly corn oil) or a HF diet in which 9% of dietary lipids was replaced by EPA and DHA (using EPAX 1050TG; Pronova Biocare, Norway). Whole body oxygen consumption, CO_2 production, and deep body temperature were analyzed in resting animals, using an indirect calorimetry system INCA (Somedic; Sweden) and telemetry system E-Mitter (Mini Mitter Co. Inc.; USA). Gene expression was assessed by qRT-PCR.
Results: In the 30°C-acclimated mice, whole body oxygen consumption was 2-fold higher at 20°C than at 30°C and it was not affected by the ectopic UCP1 in white fat. Mean values of CO_2/O_2 ratio (RQ value) were higher in the nontransgenic than in aP2-*Ucp1* mice, both at 20°C (0.883±0.016 vs. 0.847±0.017) and at 30°C (0.941±0.015 vs. 0.918±0.027) and the differences between genotypes were confirmed by the plots of RQ vs. percent relative cumulative frequency (PRCF). In accordance with the known atrophy of brown adipose tissue in the aP2-*Ucp1* mice, minimum deep body temperature (°C) was lower in the transgenic mice than in nontransgenic mice if measured at 20°C (32.7±0.4 vs. 34.3±0.2) but not at 30°C (34.7±0.7 vs. 34.8±0.2). Study of the effects of n-3 polyunsaturated fatty acids indicated strong induction by EPA and DHA of the genes engaged in lipid oxidation, CPT-1 and PPAR-alpha, in adipocytes in epididymal WAT (2.1- and 2.5-fold induction, respectively) but not in skeletal muscle or liver. In this experiment, no significant effect of the diet on either whole body oxygen consumption or RQ values was observed.
Conclusion: Modulation of WAT metabolism by ectopic UCP1 is likely to result in increased *in situ* lipid oxidation that can help to limit fat accumulation in animals exposed to HF diet and that could be detected at the whole organism level by the indirect calorimetry. However, even lower induction of fatty acid oxidation in WAT (and possibly other tissues) by dietary EPA and DHA, undetectable by indirect calorimetry, may contribute to the anti-obesity effect of these compounds.

PS 53

Inflammation and adipose tissue

0715

Serum visfatin increases with progressive β cell deterioration

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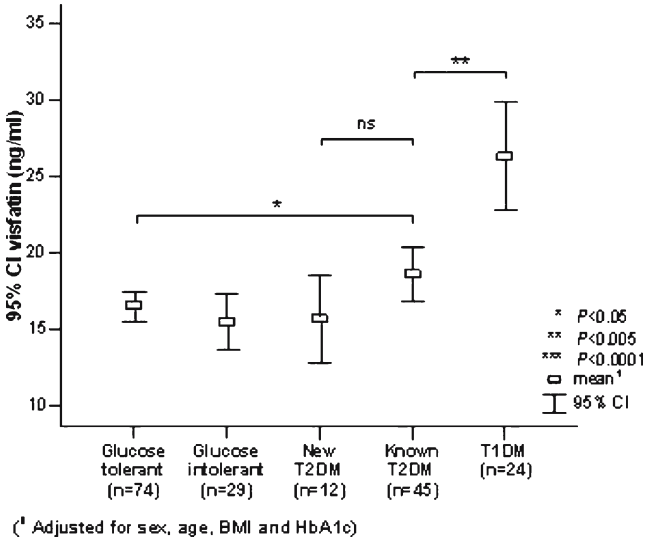
Background and Aims: Visfatin has shown to be increased in type-2 diabetes mellitus (T2DM), but to be unrelated to insulin sensitivity. We hypothesized that visfatin is associated with insulin secretion in humans.
Materials and Methods: A cross-sectional study was conducted in 103 non-diabetic (non-DM) men and 57 (31 male and 26 female) T2DM patients. Type-1 diabetic (T1DM) patients with long-standing disease (n=24; 11 male and 13 female) were also studied.
Results: In non-DM subjects, circulating visfatin (ELISA) was independently associated with insulin secretion [acute insulin response to glucose (AIRg), from intravenous glucose tolerance tests], but not with insulin sensitivity (S_I) or other metabolic or anthropometric parameters, and AIRg alone explained between 4% and 8% of visfatin variance ($\beta=-0.31$, $P=0.007$). Circulating visfatin was increased in T2DM [mean (95% CI): 19 (18–21) ng/ml *vs.* 16 (15–17) ng/ml, for T2DM and non-DM subjects,

respectively; P=0.005 (adjusted for sex, age and BMI)], although this association was largely attenuated after accounting for HbA1c (Figure). Finally, circulating visfatin was found to be increased in patients with long-standing T1DM, even after adjusting for HbA1c values [mean (95% CI): 26 (22–29) ng/ml; P<0.005 and P<0.0001 (adjusted for sex, age, BMI and HbA1c), compared to either T2DM or non-DM subjects, respectively; Figure].

Conclusion: Circulating visfatin is increased with progressive β-cell deterioration. The study of the regulation and role of visfatin in diabetes mellitus merits further consideration.



A. L-B. is a Research Investigator of the Fund for Scientific Research "Ramon y Cajal" (Ministry of Education and Science, Spain).

0716

The expression of visfatin in viceral fat of obese rats and the regulation of blockade of renin-angiotensin-system (RAS)

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Background and Aims: Adipose tissue secretes several proteins (adipokines) that may influence insulin sensitivity and atherogenesis. Visfatin is a newly identified adipokine, highly expressed in human visceral fat. Visfatin's plasma levels are increased with expanding obesity and it is thought to promote accumulation of visceral fat, most propably acting through the insulin receptor pathway. It has been demonstrated that renin-angiotensin system (RAS) relate to insulin resistance state. RAS blockade promotes the recruitment and differentiation of pre-adipocytes and that increased formation of small insulin-sensitive adipocytes counteracted ectopic deposition of lipids, thereby improving insulin sensitivity. Angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers have been reported to improve insulin sensitivity, but there are no reports on the relationship between visfatin concentrations and blockade of the renin-angiotensin system (RAS). We therefore examined the association between insulin sensitivity assessed by the euglycemic-hyperinsulinemic glucose clamp technique and visfatin mRNA levels as well as the RAS blockade on visfatin mRNA expression in high-fat diet induced insulin resistance rats.

Materials and Methods: 24 6-week-old male rats were randomly devided into 3 groups: normal control group, insulin resistance group, intervention group. Insulin resistance rat model was created by high fat high caloric laboratory chow for 8 weeks. Intervention treatment was performed by AT2 receptor antagonist Valsartn (15 mg/kg, 4 weeks). Insulin sensitivity was measured with euglycemic, hyper-insulinemic clamp technique. AGT, GLUT-4, visfatin mRNA expression levels in epididymal fat were examined by the RT-PCR.

Results: 1.**comparison between insulin resistance and normal group**: The percentages of visceral fat (epididymal fat as example) in whole body weight have a striking increase (3.5% vs. 2%). Visfatin and AGT mRNA levels were significantly increased in high-fat diet induced insulin resistance rats when compared to normal control group, whereas GLUT-4 mRNA and insulin-stimulating glucose disposal rates (GDR) during the clamp were significantly decreased (5.5 $mg/kg^{-1}*min^{-1}$ vs. 12 $mg/kg^{-1}*min^{-1}$). Fasting insulin levels were also increased compared to control group (87.78 uIU/ml vs. 15.24 uIU/ml). The fasting serum glucose levels didn't have notable difference. 2. **intervention group compared to the above two groups**: After 6 wks treatment with Valsartn, the mean fasting insulin levels reduced to 30.28 uIU/ml, GDR increased to 9 $mg/kg^{-1}*min^{-1}$. There was also a decrease of the visceral fat percentage (2.9%), yet not have significantly difference. AGT and visfatin mRNA were significantly reduced, GLUT-4 mRNA were also increased.

Conclusion: Visfatin mRNA levels were significantly increased in obese insulin resistant state and this may

related to their increased adipose tissue mass. RAS blockade can improve insulin resistance state effectively and can reduce the visfatin levels in adipose tissue.
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0717

High-fat diets do not provoke adipose tissue inflammation in obese mice when including n-3 polyunsaturated fatty acids

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Background and Aims: Inflammatory changes in white adipose tissue of obese individuals appear to have a causal role in development of insulin resistance and diabetes mellitus type 2. Based on beneficial effects of n-3 polyunsaturated fatty acids (PUFAs) on inflammatory diseases and diabetes development, we aimed at investigating possible effects of PUFAs on white adipose tissue inflammation in obese diabetic mice.
Materials and Methods: We treated obese diabetic (db/db) as well as lean non-diabetic mice (db/+) with either low-fat standard diet (LF) or high-fat diets enriched in saturated and monounsaturated fatty acids (HF/S), n-6 PUFA (HF/6) and the latter including 30% of fatty acids as n-3 PUFA (HF/3) for 6 weeks. Gene expression was evaluated from db/db and LF db/+animals by oligonucleotide microarrays (3 per group) and by quantitative RT-PCR (4–5 per group).
Results: We found increased expression of 244 genes in db/db animals treated with HF/S compared to those on LF, many of which were involved in inflammatory responses indicating macrophage infiltration (e.g., CCL2, CD68, Itgam). N-3 PUFA reverted most HF-induced changes in db/db animals. Gene expression profiles of db/+mice revealed only few HF-induced changes. Histological analyses revealed considerable mononuclear infiltrates mostly expressing the macrophage markers F4/80 and/or MAC-2 in white adipose tissue from db/db mice fed HF/S compared to those on LF. Inclusion of n-3 PUFA in the high-fat diet completely prevented macrophage infiltration induced by high-fat diets. Feeding HF/S resulted in a two-fold borderline-significant increase in JNK phosphorilation that was almost completely prevented by the n-3 PUFA containing diet in db/db mice.
Conclusion: We conclude that protective effects of n-3 PUFAs on development of complications of human obesity could be mediated by their anti-inflammatory effect in white adipose tissue.

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0718

Lamin C isoform expression is increased in subcutaneous adipose tissue in human obesity and type 2 diabetes. Relationship with inflammatory markers

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Background and Aims: Variations of gene expression in adipose tissue may be involved in obesity and related metabolic abnormalities. Recently, lamins have been associated with proteins involved in adipocyte differentiation in lipodystrophy syndromes associated with insulin resistance. However, little is known about the role of lamin in adipose tissue of obese and of type 2 diabetic (T2D) patients. In this work, we studied the expression of lamin A/C (LMNA) and adipogenic and inflammatory markers in subcutaneous adipose tissue of lean, obese, and type 2 diabetic subjects.
Materials and Methods: Subcutaneous adipose tissue samples were obtained from 52 non diabetic subjects with different degrees of obesity and from 25 subjects with T2D. Clinical and anthropometrical variables were obtained. Real Time PCR was used to measure mRNA gene expression of Lamin A and C, PPARγ, SREBP1c, CD14 and CD68 in whole adipose tissue. Likewise, variation of Lamin A and C in response to an endotoxin inflammatory stimulus was measured in subcutaneous-isolated preadipocytes and in the human monocytic THP-1 cell line.
Results: In non-diabetic subjects, Lamin C mRNA expression levels were significantly increased in the morbidly obese group compared with the lean group (8.47±0.63 vs 7.72±0.57; $p<0.01$). In the multiple linear regression analysis, Lamin C was found positively associated with BMI ($\beta=0.576$, $p<0.001$) and negatively associated with CD68 ($\beta=-0.442$, $p<0.01$). In T2D patients, Lamin C mRNA expression was increased compared with non diabetic subjects (8.67±0.44 vs 7.91±0.55; $p<0.001$). Regression analysis showed that Lamin C was positively associated with gender ($\beta=0.262$, $p<0.05$) and PPARγ ($\beta=0.317$, $p<0.01$) and negatively associated with CD68 ($\beta=-0.652$, $p<0.001$). Lamin A was found more expressed in stromovascular fraction than in mature adipocytes. Treatment of preadipocytes and *in vitro* differentiated adipocytes

with endotoxin (LPS) did not affect Lamin A/C mRNA or protein expression. On the contrary, treatment of THP-1 monocytic cell line with the endotoxin significantly increased Lamin A/C mRNA and protein expression in a dose-dependent manner.

Conclusion: Obesity and type 2 diabetes are associated with increased expression levels of Lamin C in subcutaneous adipose tissue. Our data provide for the first time evidence that LMNA mRNA expression is modulated in human obesity and T2D and lamin C is related to adipose inflammatory environment.

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0719

Adipose tissue is infiltrated with macrophages around dead fat cells in subjects with high liver fat content, independent of obesity

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Background and Aims: The causes of insulin resistance related hepatic fat accumulation are unknown. Adipose tissue of obese mice and men is characterized by increased number of macrophages potentially regulating liver fat via adipose tissue-derived factors. It is unknown whether i) number of macrophages, ii) gene expression of macrophage marker CD68 or chemokines attracting monocytes/macrophages or iii) expression of genes related to inflammation and/or insulin resistance differ in adipose tissue of equally obese humans with low or high liver fat content (LFAT). We determined in human adipose tissue: i) number of macrophages and macrophage aggregates suggested to engulf dead adipocytes (crown-like structures, CLS) ii) expression of monocyte-chemoattractant protein 1 (MCP-1) and macrophage inflammatory protein 1α (MIP-1α) and iii) adiponectin, PPARγ, TNFα and PAI-1 in BMI-matched subjects with low and high LFAT.

Materials and Methods: 20 non-diabetic apparently healthy overweight/obese women were divided into low and high LFAT groups based on their median LFAT (2.6±0.4 vs. 15.3±3.1% for low vs. high LFAT, mean±SEM, $p<0.001$) measured using magnetic resonance proton spectroscopy. Surgical adipose tissue biopsies were obtained and adipose tissue mRNA concentrations of CD68, MCP-1, MIP-1α, PPARγ, TNFα and PAI-1 and of adiponectin were measured using real-time PCR. Dead adipocytes were identified by negative perilipin staining and the number of macrophages by immunohistochemistry using antibodies against the macrophage marker CD68. In addition, fasting serum lipids, insulin and adiponectin were measured.

Results: The groups had similar BMI (35.5±1.0 vs. 36.8±0.9 kg/m^2, low vs. high LFAT) and age (42±3 vs. 38±2 yrs). The high LFAT group had higher insulin ($p=0.004$) and lower HDL cholesterol ($p=0.010$) concentration than the low LFAT group. Expression of the macrophage marker and monocyte/macrophage chemokines were significantly higher in adipose tissue in high LFAT group as compared to low LFAT group: (CD68: 3.3±0.4 vs. 2.0±0.1, $p=0.007$, MCP-1: 0.55±0.08 vs. 0.34±0.03, $p=0.037$, MIP-1α: 1.0±0.2 vs. 0.37±0.06, $p=0.007$). CD68 expression was significantly correlated with LFAT (Spearman's $r=0.57$, $p=0.009$) but not with BMI ($r=0.02$, $p=0.945$). Adipose tissue expression or serum concentration of adiponectin did not differ between the groups. The CLSs were identified around dead adipocytes (i.e. absence of perilipin) and their quantitative analyses are ongoing.

Conclusion: Adipose tissue is inflamed in those obese subjects who have increased liver fat content. Dead adipocytes may be the signal for macrophage accumulation in adipose tissue. Lack of difference in serum or adipose tissue adiponectin between subjects with high and low liver fat content does not negate a role for adiponectin in regulating liver fat but suggests additional contribution of adipose tissue-dependent or -independent mechanisms.

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0720

Gene expression profiles and roles of leukocyte cell adhesion molecules in obese-related inflammation in adipose tissue

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Background and Aims: Obesity increases the risk of developing diabetes and cardiovascular disease. Recently, obesity has been described as a "low-grade"systemic inflammatory condition. It has been reported that macrophages are increased in adispose tissues of obese people suggesting that adipose tissue itself is a source and site of

inflammation. Leukocyte recruitment into inflammatory sites is promoted by chemokines and cell adhesion molecules. Moocyte chemoattractant protein-1(MCP-1), one of the major chemokines, is upregulated in adipose tissues of obese individuals suggesting that MCP-1 contributes to the recruitment of macrophage infiltration. However, the adhesion pathway of monocytes into adipose tissue has not been clarified. We aimed to clarify the adhesion molecules which contiributes to the process of macrophage infiltration into adipose tissue using obese mouse models.

Materials and Methods: We examined glucose and lipid metabolism of 8 week-old female C57/BL6 (n=10) and db/db mice (C57BL/KsJ-db/db) (n=10). We carried out microarray gene profiling of RNA isolated from visceral adipose tissues from these mice using Affymetrix Gene Chip Mouse Genome 430 2.0 Array. After data analyses, we measured the transcript levels of selected genes based on their biological functions using quantitative real-time PCR. In addition, the distribution was examined by immunohistochemistry.

Furthermore, 7 week-old male C57/BL6 mice were fed with regular diet (n=9) and high-fat diet (HFD) (n=11) respectively for 12 weeks. We performed insulin tolerance test (ITT) and intraperitoneal glucose tolerance test (IPGTT), and then evaluated the visceral adipose tissues of these mice by microarray, real-time PCR and immunohistochemistry as described above.

Results: The db/db mice and HFD mice showed significant increase of body weight, weight of visceral adipose tissue, triglyceride, free fatty acid, blood glucose and insulin level as compared with C57/BL6 control mice. Hemoglobin A1c was not different in all groups. HFD mice showed glucose intolerance and insulin resistance by ITT and IPGTT. Microarray profilling showed that more than twice upregulated genes consisted of 1001 genes in db/db mice and 566 genes in HFD mice. We classified these genes into Gene Ontology categories. The analyses indicated the upregulation of cell adhesion-related genes including P-selectin, P-selectin glycoprotein ligand-1 (PSGL-1) and L-selectin as well as immune response-related genes in db/db mice and HFD mice. However, expression of E-selectin was not detected in all groups. Quantitative real-time PCR and immunostaining confirmed the transcriptional and protein level upregulation of these adhesion molecules. Macrophage-related genes such as CD14 were also increased in adipose tissues of db/db and HFD mice.

Conclusion: Current results indicate that low grade inflammation is occurred in visceral fat of obese mice with insulin resistance. PSGL-1 is expressed on leukocyte surface and acts as a ligand for P-selectin which is expressed on endothelial cells. PSGL-1 also binds to E- and L-selectin. Selectin-PSGL-1 pathway may promote the recruitment of macrophages into adipose tissue. Selectin and PSGL-1 might be a novel target for the prevention of diabetes and atherosclerosis in obese people.

0721

Obesity and role of adipose tissue (AT) in inflammation and insulin sensitivity - the GENOBIN study

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Background and Aims: Obesity is characterized as a low-grade inflammation state shown as increased circulating concentrations of several cytokines and acute phase proteins. AT has been illustrated to secrete a wide number of inflammatory factors. Recently serum amyloid A (SAA) was found to be expressed in AT. There is also the growing view that the obesity-associated inflammatory state plays a causal role in the development of type 2 diabetes. The aim of the present study was to examine circulating inflammatory markers and their gene expressions in AT in obese subjects with features of metabolic syndrome.

Materials and Methods: Seventy-five overweight to obese subjects (BMI 32.9±2.8 kg/m2, mean±SD) with impaired glucose tolerance and other features of metabolic syndrome, aged 60±7 years participated in the lifestyle modification intervention for 33.3±1.1 weeks. Fasting blood samples were drawn before the intervention to determine serum glucose, lipids, lipoproteins, cytokines, CRP and SAA. SAA gene expression was studied from mRNA extracted from abdominal subcutaneous AT using real-time PCR. The study was approved by the Ethics Committee of the Kuopio University Hospital and was performed in accordance with the standards of the Helsinki Declaration.

Results: In women the serum SAA concentration (17.6±15.7 ug/ml in women vs. 8.7±4.9 ug/ml in men, p=0.002) and mRNA expression in AT (108±71 in women vs. 26±19 in men, p=0.001) were significantly higher than in men. In the whole study population serum SAA concentrations correlated positively with percentage of body fat (r2=0.502, p=0.001), body fat mass (r2=0.443, p=0.001) and BMI (r2=0.448, p=0.001) in baseline. Interestingly, we found also significant correlation between leptin and fS-SAA (r2=0.518, p=0.0001) and CRP (r2=0.309, p=0.007).

In women CRP and fS-SAA were negatively associated with insulin sensitivity (r2=−0.388, p=0.021; r2=−0.355, p=0.039, respectively). Serum levels of CRP also correlated highly with fS-SAA (r2=0.529, p=0.001). Women were also characterized by larger adipocytes than men (p=0.0001), but the size of adipocyte did not correlate with inflammatory markers.

Conclusion: The present study shows that elevated serum concentrations of inflammatory markers are involved in the obesity-induced inflammatory state. The relationships between leptin and inflammatory markers indicate that leptin may have a role in the regulation of the obesity-associated inflammation. Furthermore, the associations of inflammatory markers with insulin sensitivity confirm their contribution to the development of type 2 diabetes.

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0722

Mesothelial cells as a source of the pro-inflammatory cytokine IL-18 in human omental fat

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Background and Aims: In obesity, the production of pro-inflammatory cytokines by adipose tissue is proposed to participate to the impairment of insulin sensitivity. The objective of this study was to analyze the expression profile of pro-inflammatory cytokines and cofactors in the visceral and the subcutaneous fat of obese subjects.

Materials and Methods: Omental (OAT) and subcutaneous adipose (SAT) samples were collected during a gastroplasty surgery in 14 obese female patients (BMI=47.5±1.6 kg/m^2) having no associated co-morbidities. RNA from each samples were prepared and cRNA were hybridized on Affymetrix GeneChip containing almost 39,000 transcripts. Tissue samples were fixed in paraformaldehyde for protein detection by immonuhistochemistry.

Results: Among the 24 pro-inflammatory cytokines and cofactors found to be differentially regulated in both tissues, 19 were up regulated in OAT as compared to SAT. The interleukin 18 (IL-18) was the pro-inflammatory cytokine the most up regulated in OAT as compared to SAT (+6 fold). This cytokine was mostly expressed in the stromavascular fraction of OAT suggesting that other cells than adipocytes preferentially expressed IL-18. The analysis of cellular location of IL-18 protein expression demonstrated that this interleukin was expressed in neutrophils and in mast cells, both in OAT and SAT, but not in macrophages. In OAT, mesothelial cells were identified as an additional source of IL-18. Expression of this pro-inflammatory cytokine in mesothelial cells, known to be absent in SAT, may explain the higher level of IL-18 expression in OAT.

Conclusion: Altogether, our data demonstrate that 1) in obese subjects the omental fat depot had a higher expression level of pro-inflammatory cytokines than in SAT, and 2) in OAT, mesothelial cells, by secreting IL-18, participate to the inflammatory process associated to obesity.

0723

TNF-alpha activates the expression of inflammatory cytokines in undifferentiated human pre-adipocytes

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Background and Aims: The adipose tissue is not only important for lipid storage and release; it is also the largest endocrine and secretory organ of the body releasing a number of important adipokines like leptin, adiponectin and retinol-binding protein-4 (RBP-4) as well as cytokines like Interleukin-6 (IL-6) and monocyte chemo-attractant protein-1 (MCP-1) that are related to inflammation and the innate inflammatory response. Moreover, the adipokines cross-talk with other tissues of the body to modulate insulin sensitivity and action. Type 2 diabetes and obesity are characterized by low-grade inflammation with elevated levels of acute phase-reactants like CRP and serum amyloid A. This inflammatory response is believed to be initiated in the adipose tissue and also to play an important role for the development of cardiovascular disease.Several studies have shown that it is not the primary adipocytes, which are the major producers of cytokines in the adipose tissue but rather the stromal cell fraction containing preadipocytes, fibroblasts, endothelial and inflammatory cells. During the development of obesity and insulin resistance, macrophages are recruited into the adipose tissue and this seems to induce a sequence of events, which are important for the development of insulin resistance and probably also the inflammation.Here we examined if the undifferentiated preadipocytes in human adipose tissue are responsive to inflammatory cytokines, in particular TNF alpha, since this potent cytokine is released by infiltrating macrophages.

Material and Methods: Abdominal subcutaneous adipose tissue was obtained from six different donors by needle biopsy. From the biopsy, the stromal vascular fraction was

retrieved following collagenase digestion and removal of the primary adipocytes. The inflammatory cells were also removed by magnetic bead eluation of CD 14 and CD 45 positive cells. The remaining part of the fraction was seeded and grown in six-well plates. The cells were either left untreated or grown in the presence of TNF-alpha, IL-6 or Resistin for 10 days. RNA was extracted with the QIAGEN RNEasy mini kit. The gene expression was analyzed with real-time RT-PCR (Taqman).

Results: The undifferentiated preadipocytes expressed several inflammatory cytokines also in the non-stimulated state. However, the expression of TNF-alpha itself as well as IL-6 and MCP-1 were markedly and significantly increased in the presence of TNF-alpha. Interestingly, CCR-2, the receptor for MCP-1, was not expressed in human preadipocytes indicating that these cells produce MCP-1, as well as other cytokines, but they are not target cells of MCP-1. Both IL-6 and Resistin had only minor inflammatory effects when compared to TNF-alpha.

Conclusion: These data show that the presence of TNF-alpha, which is released by macrophages, leads to a marked inflammatory phenotype in human undifferentiated preadipocytes. This finding provides a mechanism for the increased production of cytokines found in the stromal vascular cells of the adipose tissue in obesity. Furthermore, since TNF-alpha is not released by adipocytes, these data support a key role for invading macrophages in inducing the inflammatory response in human adipose tissue in obesity.

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0724

Insulin exerts an anti-inflammatory effect in adipose cells by antagonising IL-6 signaling

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Background and Aims: Both obesity and insulin resistance correlate with a low-grade chronic inflammation that probably originates in the adipose tissue. Several studies have in recent years shown that cytokines, such as interleukin-6 (IL-6), secreted by the adipose tissue constitute an inhibitory effect on the insulin signaling, suggesting a role in the development of insulin resistance. To further explore the cross-talk between IL-6 and insulin signaling pathways we have studied the effect of insulin on the IL-6 signaling pathway.

Materials and Methods: The mouse pre-adipocyte cell line, 3T3-L1, was differentiated to mature adipocytes and incubated with insulin, IL-6 and IL-6 together with insulin. The same experiments were performed in MEF cells lacking functional SHP2. Protein and RNA samples were purified and analyzed by western blotting and real-time PCR (TaqMan) analysis, respectively.

Results: Here we show a mechanism whereby insulin reduces the tyrosine phosphorylation and induces the serine phosphorylation of STAT3 and, thereby, reduces its nuclear localization and transcriptional activity. As a consequence, expression of the inflammatory markers, serum amyloid A 3 (SAA3) and haptoglobin, are significantly decreased in cells stimulated with both IL-6 and insulin compared to cells treated with IL-6 alone. We further show that the protein tyrosine phosphatase, SHP2, is activated upon insulin signaling. Since SHP2 is required for dephosphorylation of STAT3, this finding provides a mechanism for the effect of insulin on the tyrosine phosphorylation of STAT3. Additionally, we present that the effect of insulin on the IL-6 signaling pathway is mediated through the MAPK pathway, since treatment with the MEK inhibitor, PD98059, reduces all of the observed effects. We, therefore, conclude that insulin exerts an important anti-inflammatory effect on IL-6 signaling.

Conclusion: We conclude that insulin, through the MAPK-signaling pathway also involving SHP2, exerts an anti-inflammatory effect by anagonizing IL-6 signaling.

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PS 54

Prediction of risk in insulin resistance and diabetes

0725

Simple risk factors for developing type 2 diabetes mellitus as identified in the STOP-NIDDM trial

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Background and Aims: Interventions to prevent or delay type 2 diabetes mellitus (DM) should be available for subjects at increased risk. The risk may be assessed by specific characteristics, so called risk factors that are associated with the development of the disease. Ideally, such risk factors are causal, and may favorably be altered by interventions and are relatively easy to determine even in a general practice setting.
Materials and Methods: The STOP-NIDDM trial showed that acarbose treatment reduced the individual risk of developing DM in patients with impaired glucose tolerance (IGT). The trial database (n=1160) was used to select important predictors by means of Cox regression for the time to onset of DM. The final model was externally validated using DECODE study data.
Results: Sex, body height (>168 vs.≤168 cm), waist circumference (≤88 vs. 89-102 vs.>102) and diagnosed hypertension were identified as both important and readily available risk factors. The 2.5-year risk of developing DM for each combination of these four risk factors was calculated based on the full Cox model and is presented in the following table.

Risk chart for 2.5-year risk of developing type 2 diabetes mellitus

	Women											
Body height (cm)	>168						≤168					
Waist circumference (cm)	≤88		89–102		>102		≤88		89–102		>102	
Diagnosed hypertension	no	yes	no	yes	no	yes	no	yes	no	yes	no	yes
2.5-year-risk (%)	15	19	17	23	24	31	22	29	27	34	36	44
	Men											
Body height (cm)	>168						≤168					
Waist circumference (cm)	≤88		89–102		>102		≤88		89–102		>102	
Diagnosed hypertension	no	yes	no	yes	no	yes	no	yes	no	yes	no	yes
2.5-year-risk (%)	17	22	20	26	28	35	26	33	31	38	41	50

Conclusions: Based on simple risk factors such as sex, body height, waist circumference and diagnosed hypertension patients may be screened at low costs. Patients at increased risk for developing DM may be offered further diagnostic procedures and/or specific DM prevention strategies, such as lifestyle modification with or without pharmaceutical intervention (i.e. acarbose or metformin).
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0726

Use of clinical parameters to identify insulin resistance, measured by euglycaemic hyperinsulinaemic clamp, in an Italian population of 300 subjects

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Background and Aims: Insulin resistance is associated with adverse outcomes, such as cardiovascular disease and type 2 diabetes mellitus. However, the methodologies currently available for the measurement of insulin resistance are cumbersome to administer and expensive. Given the relative ease of measuring plasma glucose and insulin, numerous alternative indexes, both fasting and during oral glucose tolerance test (OGTT), have been proposed. The EGIR database, "enlarged" with the insulin resistant population of PIMA Indians and Mexican Americans, is the only one presently existing. Aim of the present study was to investigate the reproducibility of these data in a population of about 300 Italian subjects, evaluated by OGTT and euglycemic hyperinsulinaemic clamp study (40 mU/m^2.min).
Materials and Methods: 101 males and 214 females, mean age: 37.7+4.2 years, 10 unknown diabetic subjects, 31 IFG and/or IGT, 254 normo-tolerant subjects). The proposed definition (Diabetes 2005, 333) of M <5.04 mg/min.kg of lean mass identified as insulin-resistant only 5% of normal and 25% of diabetic subjects, whilst the surrogate formula (BMI>28.9 kg/m^2 or HOMA-IR>4.65 o BMI>27.5 and HOMA-IR>3.60) identified as insulin-resistant the 44% of normo-tolerant subjects.
Results: In order to modify these indexes for the Italian population, we considered all diabetic and IFG/IGT subjects as insulin-resistant, and an M value equal to mean+ 1DS (6.4 mg/kg.min) of the subjects was chosen as cut-off value for insulin resistance. We therefore calculated which index, among those proposed in the literature, (Matsuda, Quicki, BMI, fasting insulin, HOMA, metabolic syndrome, according to NCEP-ATPIII criteria) is able to predict insulin resistance, measured by euglycemic clamp, with the best sensitivity and specificity. We found that Matsuda index, combined with metabolic syndrome, displays a good sensitivity (63.4%) and an excellent specificity (91.3%); among the fasting indexes, fasting insulin combined with metabolic syndrome criteria, showed the best sensitivity and specificity (67.1% and 83.1%, respectively).

Conclusion: In conclusion, parameters determined on EGIR enlarged with an insulin resistant population seems irreproducible on Italian population. On the contrary, Matsuda index combined with NCEP criteria for metabolic syndrome has the best predictive value for insulin resistance, evaluated by insulin clamp. In the absence of OGTT, the most predictive index is the combination of fasting insulin and metabolic syndrome.

0727

Elevated concentrations of asymmetrical dimethylarginine predict deterioration of glucose tolerance in women with previous gestational diabetes mellitus

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Background and Aims: Women with previous gestational diabetes (GDM) have a high risk for type 2 diabetes mellitus. The endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine (ADMA) could be related to disorders of glucose metabolism such as insulin resistance. To evaluate if ADMA predicts a deterioration of glucose tolerance in women with previous GDM and to assess the time course of concentration changes we analysed ADMA in women with previous GDM 14–16 weeks after delivery and after a median follow-up of 2.8 years (interquartile range: 1.5–4.6).
Materials and Methods: ADMA and L-arginine were determined by high performance liquid chromatography in 77 women with previous GDM who underwent also an oral glucose tolerance test. Survival analysis was performed using the Kaplan-Meier method and Cox regression analysis.
Results: Deterioration of glucose tolerance was observed in 36% of women with ADMA above and 11% of those with ADMA below the median of 0.56 μmol/l (P=0.008, log-rank test). ADMA correlated significantly with mean arterial blood pressure (MAP) but not with other parameters. The fully adjusted hazard ratio for a decline of glucose tolerance during follow-up was 4.06 (95% CI: 1.13–14.54; P=0.031; adjusted for age, BMI, fasting glucose, insulin sensitivity, MAP, high sensitivity C-reactive protein [hsCRP] and creatinine). Apart from ADMA, hsCRP also predicted deterioration of glucose tolerance [hazard ratio 3.69 (1.01–13.45, P=0.048)]. L-arginine was not associated with changes of the glucose tolerance status. ADMA decreased significantly during follow-up.
Conclusion: High ADMA concentrations after delivery predict deterioration of glucose tolerance in women with previous GDM.
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0728

Do patients with elevated blood glucose and the metabolic syndrome have increased risk of diabetes type 2 according to Finnish Diabetes Risk Score criteria?

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Background and Aim: Finnish Diabetes Risk Score (FRS), simple, and quastionaire based tool was developed to identify individuals at high risk of diabetes. This tool has not been used and validated in Polish population yet. Similarly the relation between the Metabolic Syndrome (MS) - cluster of metabolic disorders known to be related to diabetes and CVD, and FRS has not been studied.
The aim was to study the association of elevaed blood glocose, the MS and the risk of development of diabetes type 2 according to FRS.
Sample and Methods: The studied population were 977 men and 983 women aged 45–64, randomly selected from the Krakow's population. All analyses were performed after exclusion of subjects with known diabetes. The MS was calculated according to the Interational Diabetes Federation criteria. Categories of FRS were made according to definition. The chi square test was used for estimation of the relation of the elevated glucose and the MS in FRS categories.
Results: FRS higher that 7 was present in 66,8% men and 74,1% women. Fasting blood glucose over 6,1 mmol/l was observed in 16,8% men and 9,7% women. Fasting blood glucose over 7 mmol/l had 4,8% men and 3,0% women. The metabolic syndrome was present in 30,3% men and 32,4% women. The frequency of low and high FRS in groups with elevated glucose and MS are presented in the table.

	Glucose >6,1 mmol/l		Glucose >7,0 mmol/l		MS (IDF)	
	Men*	Women*	Men*	Women*	Men*	Women*
>7	3,4%	2,3%	0%	0%	1,5%	0,7%
15–20	37,7%	62,1%	45,2%	74,1%	29,7%	34,9%

* p<0,05

Conclusion: Low FRS (less than 7) is very rare in the subjects with elevated glucose and with the MS. Only one third of men and women with the MS have high FRS (15–20). The subjects with elevated glucose had high incidence of FRS between 15–20, however this association was stronger in women than in men. Further investigations on the defition of the MS and prognostic tool for prediction of diabetes in everyday practice should be carried on.

0729

Reduced antioxidative defence and prothrombogenic factors in children and adolescents with metabolic syndrome

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Background and Aims: Since early glycoregulation disorders, impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) are associated with metabolic syndrome (MS), the aim of this study was to determine the frequency of metabolic syndrome, examine the extent of disorders, and analyze insulin sensitivity, secretion, lipid status, prothrombogenic factors and antioxidative status. Three of the following five criteria were used for MS diagnosis in children and adolescents: waist circumference≥90 percentile for age and sex; triglycerides>1.7 mmol/l; HDL cholesterol <1.0; hypertension≥90 percentile for height, age and sex; glycaemia>6.0.
Materials and Methods: The study included 24 obese individuals (age 10–20 years, BMI or waist circumference≥90 percentile). Oral glucose tolerance test (OGTT) was used to evaluate the extent of disorder. Insulin sensitivity was determined by HOMA IR, insulin secretion by HOMA β, lipid status by spectrophotometry (total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides). Prothrombogenic factors analyzed were the plasma plasminogen activator PAI-1 (by plasminogen substrate assay) and fibrinogen. Erythrocyte dismutase (SOD) and erythrocyte glutation peroxide (GPX) were markers of antioxidative defense.
Results: Metabolic syndrome was found in one third of patients (BMI 33.9±0.74 kg/m^2; waist circumference 98±0.55 cm; blood pressure 123.7±4.8/78.7±13.1 mmHg; insulinemia mean value 57.7±20.3 mlU/l; HOMA IR elevated in all patients (6.5±3.2); HOMA β showing insulin secretion disorder (246±55.6); increased triglycerides (1.77±0.05 mmol/l); decreased HDL (0.72±0.12 mmol/l)). These patients had increased values of PAI-1 (6.4±0.08 U/ml), normal values of fibrinogen, and reduced antioxidative defense (SOD 1083.7±0.40 U/gr/Hgb; GPX 30±0.82 U/gr/Hgb). The other two thirds showed 1–2 metabolic syndrome criteria (mostly waist circumference 93±21.6 cm; decreased HDL 1.0±0.15 mmol/l), insulin resistance parameters (HOMA IR 6.2±4.7, insulin mean value 70.0±37.9 mlU/l), normal prothrombogenic status and reduced antioxidative defense (SOD 932±0.81 U/gr/Hgb; GPX 30.3±0.8 U/gr/Hgb).
Conclusion: These results indicate a growing occurrence of MS in children and adolescents and therefore an increased risk of obtaining diabetes mellitus type 2. Increased HOMA IR and HOMA β and insulinemia mean value confirm hyperinsulinism, insulin resistance and decreased insulin sensitivity, all these correlating with waist circumference and lipid status. Increased values of plasminogen and reduced antioxidative defense, all resulting in accelerated atherosclerosis and coronary disease in MS patients.

0730

Leisure-time physical activity is associated with the metabolic syndrome in type 1 diabetes; interaction with the Pro12Ala variant of the *PPAR*γ Gene (The Finn-Diane Study)

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Background and Aims: Recently, we showed that the metabolic syndrome (MetS) is frequently found in type 1 diabetes (T1D). Additionally, the MetS is associated with micro- and macrovascular diabetic complications. Physical activity has been shown to reduce the risk for MetS in the general population. Further, the Pro12Ala variant of the *PPAR*γ gene has been linked to components of the MetS (hyperglycaemia, dyslipidaemia and obesity). The aim of our study was to investigate if leisure-time physical activity (LTPA) and/or *PPAR*γ Pro12Ala genotype are associated with MetS in T1D.
Materials and Methods: MetS was defined according to the NCEP ATPIII criteria. A MetS-score (1–5) was established according to the number of fulfilled criteria, all patients having hyperglycaemia. A minimum of three criteria out of five indicated MetS. LTPA was assessed with a validated, quantitative questionnaire and expressed as MET*h/week (MET=metabolic equivalent). LTPA <10

MET*h/week indicated sedentary LTPA habits. All T1D patients in the FinnDiane study with complete data on LTPA and MetS were studied. Excluding patients with end-stage renal disease and/or major cardiovascular events, 1104 patients remained (male/female:520/584, age 36.1±11.5 yrs, duration of diabetes 20.8±11.9 yrs, BMI 25.0±3.2 kg/m^2, HbA_{1c} 8.3±1.4%). Genotyping of the Pro12Ala variant of *PPARγ* was performed using TaqMan® technology; 881 patients with data on LTPA and MetS were genotyped.

Results: MetS was found in 29.6% of the patients (male/female=28.1/31.0%, P=0.29). The genotype distribution of *PPARγ* Pro12Pro:Pro12Ala:Ala12Ala was 598:261:22 patients. 31.4% of Ala-carriers and 30.4% with the Pro12Pro genotype had MetS (P=0.76). In sedentary vs. more physically active patients, MetS was present in 35.1% vs. 27.9%, respectively (P=0.033 adjusted for age). Level of LTPA was inversely associated with the MetS-score. Stratifying for *PPARγ* genotype, the association was apparent only in Ala-carriers (see table: LTPA values are presented as MET*h/week, median[IQR]).

Conclusion: LTPA is associated with features of the metabolic syndrome in T1D. The data further suggest a gene-environment interaction as Ala-carriers of the *PPARγ* gene may respond better to physical activity than patients with the Pro12Pro genotype. Whether higher level of LTPA may lead to a reduction in the risk for diabetic late complications has to be established.

LTPA (MET*h/week, median[IQR]) by MetS-score.

MetS-score	All Patients	Pro12Pro	Pro12Ala+ Ala12Ala
1	20.0[11.0–35.0], N=259	21.0[11.0–37.0], N=135	23.0[13.0–35.3], N=66
2	22.0[10.0–35.0], N=518	21.0[9.5–34.0], N=281	22.0[11.0–35.0], N=128
3	18.0[10.0–35.0], N=239	19.0[10.0–36.0], N=132	16.0[9.0–26.0], N=67
4	14.0[7.0–33.0], N=71	14.5[4.3–39.8], N=40	10.0[4.0–16.5], N=17
5	12.0[5.5–20.5], N=17	12.5[7.3–23.5], N=10	9.0[1.5–31.0], N=5
P-value	0.017	0.356	0.0011
P-value adj. for age	0.002	0.429	0.0000036

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0731

Independent measures of insulin secretion and insulin sensitivity during the same test for clinical purposes: The Glucagon-Insulin Tolerance Test (GITT)

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Background and Aims: To validate a test for the independent assessment of insulin secretion and insulin sensitivity during the same occasion for future use in phenotyping studies for clinical use, i.e. a glucagon-C-peptide test followed by an insulin tolerance test (GITT).

Materials and Methods: The GITT measures C-peptide response to 0.5 mg of glucagon i.v. followed 30 min later by a 0.05 U/kg insulin tolerance test (ITT). Nine subjects with normal glucose tolerance participated on different days in an ITT, glucagon-C-peptide test, ITT followed by a glucagon-C-peptide test and glucagon-C-peptide test followed by an ITT (GITT) to establish whether and how the two tests can be combined. The test was then repeated to investigate the reproducibility and was validated against Botnia clamp in patients with normal and abnormal glucose tolerance and in patients with type 2 diabetes. The Botnia clamp measures the first phase insulin response to 0.3 g/kg glucose i.v. (FPIR) and insulin sensitivity (M-value) from a 2 hr euglycemic clamp started 60 min after the glucose bolus.

Results: The K_{itt} from the GITT was reproducible (CV 13% in NGT, 22% in T2D) and correlated with the M-value from the Botnia clamp (r=0.86; p=0.01, in T2D+NGT+IGT, n=20). The relative C-peptide response to glucagon correlated with FPIR (r=0.78, p=0.02).

Conclusion: The Botnia clamp provides reproducible and independent estimates of insulin sensitivity and insulin secretion on the same occasion for metabolic studies. The GITT is a pheasible and reproducible test, which provides similar information for clinical use.

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0732

A robust simple clinical measurement to diagnose partial lipodystrophy

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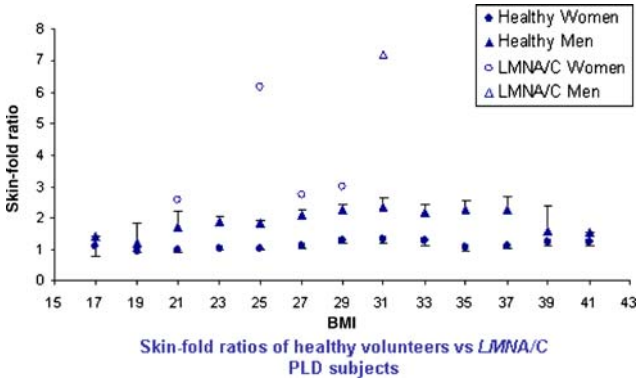
Background and Aims: Subjects with partial lipodystrophy (PLD) display the full spectrum of symptoms of the metabolic syndrome, including severe insulin resistance (IR), hypertension, hypertriglyceridaemia, low HDL-cholesterol, and hyperglycaemia. These subjects exhibit a substantial reduction in peripheral fat mass, yet show similar and often more extreme metabolic characteristics than obese individuals.

PLD is considered a rare cause of IR and type 2 diabetes, with the exception of subjects with human immunodeficiency virus, particularly those on highly active antiretroviral therapy. However, we recently started actively identifying subjects with PLD within our clinics. It appears that the frequency of PLD is possibly under-reported, as we have identified 23 subjects with irrefutable clinical characteristics of PLD amongst subjects attending T2DM, Lipid, and/or polycystic ovarian syndrome clinics within 18 months.

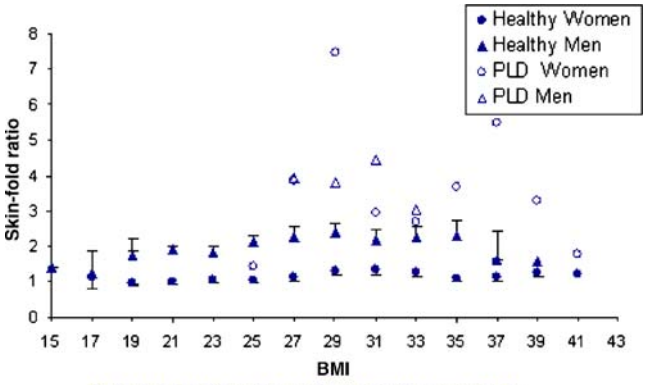
The difficulty in detecting subjects with PLD has, at least in part, been due to lack of simple clinical tests to aid diagnosis. In view of the clear phenotype of PLD, we hypothesized that a simple ratio of central to peripheral skin fold measurements would distinguish PLD from healthy individuals.

Materials and Methods: The sum of two trunk skinfolds measurements (subscapular+suprailiac) divided by the sum of two peripheral (triceps+biceps) were measured in 5 subjects with *LMNA/C* PLD, 14 subjects with PLD awaiting genetic diagnosis, and 835 healthy population-based controls aged 30–50 yrs from the Oxford Biobank.

Results:



Skin-fold ratios of healthy volunteers vs *LMNA/C* PLD subjects



Skin-fold ratios of healthy volunteers vs PLD subjects awaiting genetic diagnosis

All PLD subjects were well outside (above) the 95% confidence interval of body mass index normalised healthy subjects.

Conclusion: We demonstrate that the measurement of skin fold ratios is a robust and simple clinical tool to identify PLD. This will simplify the detection of subjects with PLD.

0733

Preservation of beta cell secretion in T1DM subjects with low risk HLA genotype

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Background and Aims: Type 1 diabetes (T1DM) is a autoimmune disease leading to the destruction of beta cells and consequently to the need of insulin therapy. HLA complex constitutes the most relevant genetic region contributing 50% of the inherited risk for T1DM.

Although previous studies demonstrated that HLA susceptible genotypes are important in determining of residual beta cell function as measured by C-peptide levels, the relationship between HLA class II and beta cell function did not lead to conclusive reports. The aim of this study was to investigate in T1DM patients the relation between HLA and differences in C-peptide levels at diagnosis and twelve months thereafter following intensive insulin therapy.

Materials and Methods: A total of 120 T1DM consecutive subjects (age 1–49; M 53%, F 47%) were studied. These subjects included those diagnosed in childhood, adolescence and young adult age. HLA class II (DRB1 and DQB1) genes were typed using a SSO (Sequence Specific

Oligonucleotide). HLA genotypes were classified in three risk categories based on the absolute risk values (AR) previously estimated in the Italian population: high risk (RA=1:23), moderate risk (RA=1:150) and low risk (RA=1:1100). C-peptide levels at diagnosis and at 12° month were measured by a radioimmunoassay validated trough the international C-peptide workshop. The normal range of fasting C-peptide established in 150 control subjects 5–40 years old, median 18 years, was 0.35–1 nm/L with intra-coefficient and inter-coefficients varying between 10 and 15%, respectively.

Statistical analysis was performed using multiple linear regression. Δ-CP, calculated as CP 12° month - CP 0° month, was $\log_{10}$ transformed to normalize its distribution.

Results: We observed that the Δ-CP showed an increasing linear trend with lower values in patients with high risk genotype, intermediate in moderate risk genotype and higher values in low risk genotype (Δ-CP=−0.25, −0.09 and 0.13 respectively; p for trend=0.009). Moreover, we did not observe any statistically significant difference when we analysed the relation between Δ-CP and age of onset (p=0.569).

Conclusion: The results of this study indicate that a preservation of beta-cell secretion occurs in T1DM subjects with low risk HLA genotype compared to the other two HLA risk categories irrespective of age at disease of diagnosis. This finding is of particular relevance for designing trials of prevention of beta cell destruction in T1DM.

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PS 55

Markers of insulin resistance and metabolic syndrome

0734

Inflammatory parameters in type 2 diabetics and in the metabolic syndrome

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Background and Aims: to investigate which inflammatory parameters seem to prove the inflammatory origin of the metabolic syndrome.

Materials and Methods: 1./19 patients who fit at laest 3 criteria - due to IDF - of the metabolic syndrome, but were glucose tolerant (9 females, 10 males: mean age: 43±16 years, BMI:32±4,8, waist circumference: in males: 110,4± 11,5 cm, in females: 93±8,5 cm).2./113 type2 non-insulin treated diabetics, (males: 65, females: 48, mean age:55± 10 years, BMI: 29±4.5, waist circumference:105± 13 cm). Clinical data: family anamnesis, waist circumference, RR, therapy. Laboratory investigations: se insulin level, HbA1c, hsCRP with immun turbidity, ferritin by sandwich-immunoassay, TNFα, IL-6, IL-6sR by ELISA. Statistical methods: Pearson correlation, ANOVA variance analysis, Mann-Whitney test, significance: p<0,05.

Results: In the metabolic syndrome group: we found significant correlation (p<0,05) between BMI and following inflammatory markers: hsCRP, IL-6sR, IL-6 and TNFα. Similar significant correlation could be found between blood sugar, HbA1c and IL-6sR. However we couldn't prove any significant link between metabolic parameters and ferritin levels, but the tendency (160±122 μg/l) was increased. HbA1c in the type2diabetic group presented correlation with hsCRP, IL-6sR and with the number of white blood cells. The blood sugar level showed correlation with the IL-6sR level. To compare these two patient groups, the level of TNFα did not presented any significant difference. The IL-6 was significantly higher in the diabetic group (p=0,02). However the level of IL-6sR was higher in the metabolic syndrome group (499±150 pg/ml) but significant difference could not be demonstrated (p=0,332).

Conclusion: To analyse our results we can state that among normal glucose tolerant metabolic syndrome patients the inflammatory markers- hsCRP, IL-6,IL-6sR, TNFα -prove the correlation between metabolic alterations and inflammation. The ferritin values on a significant level could not prove this correlation but there was a strong elevated tendency in the metabolic syndrome group. For more objective evaluation further investigations are needed with enrolling numerous patients mainly in the normal glucose tolerant metabolic syndrome group.

0735

C-reactive protein levels in subjects at risk for diabetes and its association with metabolic parameters

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Background and Aims: Subclinical inflamation is considered as a nontraditional risk factor for cardiovascular disease. By this time, there are several reports that type 2 diabetic subjects have higher serum levels of C-reactive protein (CRP) than those of non-diabetic subjects. We

evaluated CRP levels and its association with metabolic parameters in a group of patients at high risk for diabetes.
Materials and Methods: In this study we evaluated 46 patients at risk for diabetes, classified using the criteria described by Diabetes Prevention Program Research Group, and 22 control subjects matched for age, sex and body mass index. Anthropometric measurements, biochemical analyses and insulin and CRP levels were performed in each subject. Insulin resistance was assessed using the homeostasis model assessment of insulin resistance (HOMA).
Results: CRP levels were significantly higher in subjects at risk for diabetes than control subjects (3.9±1.0 vs. 2.1±0.8 mg/L, p<0.001). Subjects at risk for diabetes were more insulin resistant than control subjects (2.8±0.6 vs. 1.09±0.4 mg/L, p<0.001). CRP levels were correlated positively with systolic blood pressure (r=0.256, p=0.035), fasting blood glucose (r=0.417, p=0.001), postprandial glucose levels (r=0.367, p=0.002), total cholesterol levels (r=0.383, p=0.001), LDL levels (r=0.398, p=0.001), triglyceride levels (r=0.240, p=0.049) and HOMA score (r=0.326, p=0.007), and correlated negatively with HDL levels (r=−0.256, p=0.035). These correlations were still of significant importance after adjustment for body mass index. There was no correlation between CRP and diastolic blood pressure (r=0.160, p=0.193) and waist circumference (r=0.160, p=0.193).
Conclusion: These findings support that serum CRP level is higher in subjects at risk for diabetes than subjects wihout known risks. CRP level as an indicator of subclinical inflammation is correlated with risk factors of cardiovascular diseases and may be useful to predict accelerated atherosclerotic process in subjects at risk for diabetes.

0736

Gender as an important determinant of elevated C-reactive protein levels in subjects with the metabolic syndrome

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Background and Aims: Although there are a lot of studies on the relationship between the metabolic syndrome (MS) and C-reactive protein (CRP), a sensitive systemic marker of inflammation, little is known about gender differences. The aim of this study was to investigate the relationship between the MS and CRP in men and women.
Materials and Methods: The study population included 720 patients (354 men and 366 women) aged 40–75 years recruited from the Risk Factor in Impaired Glucose Tolerance for Atherosclerosis and Diabetes (RIAD) study. CRP serum levels were measured by an immunoturbidimetric assay. MS was defined using the IDF-criteria (2005) and was present when a person have central obesity (defined as waist circumference≥94 cm for men and≥80 cm for women) plus any two of the following factors: triglyceride levels≥1.7 mmol/l, HDL cholesterol <1.03 mmol/l in men and <1.29 mmol/l in women, blood pressure≥130/85 mmHg, and fasting plasma glucose≥5.6 mmol/l or previously diagnosed type 2 diabetes. Subjects with CRP levels greater than 10 mg/l were excluded from the analysis.
Results: Both men and women with the MS had elevated CRP levels compared to those without the MS (geometric mean (standard error) CRP: men 1.3 (0.15) vs 1.86 (0.14)mg/l, women 1.49 (0.13) vs 2.69 (0.14)mg/l; both p<0.001). There was no significant difference in CRP levels between men and women without the MS, but compared to men with the MS women with the MS had significant higher CRP levels (1.86 (0.14) vs 2.69 (0.14) mg/l, p=0.001). All geometric means were adjusted for age, smoking status and alcohol consumption. Excluding all women taking hormone replacement therapy did not change these findings. Thirty-one percent of the women who had MS also had CRP levels>3 mg/l, characterizing subjects with high cardiovascular risk, in contrast to only 15.5% of the men. The odds ratio for CRP levels>3 mg/l was 1.37 (95%CI: 0.74–2.55) for men and 3.57 (95%CI: 2.04–6.25) for women with the MS.
Conclusion: These results indicate that the MS is associated with elevated CRP levels in both genders. However there exists a strong gender difference with higher CRP levels in women with the MS whereas no gender difference was found in people without MS.

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C-reactive protein, degree of glucose tolerance and metabolic syndrome

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Background and Aims: To investigate the relationship between CRP, degree of glucose tolerance and Metabolic syndrome (MS).
Materials and Methods: Population based, cross sectional nationwide survey (n=1933 individuals, aged 35–74 years, 47% males (M), 53% females (F)). *Anthropometric parameters*: Body mass Index (BMI), Waist Circumference (WC),

Waist/Hip ratio (W/H), Systolic (SBP)/Diastolic Blood Pressure (DBP). *Laboratory parameters*: Oral glucose tolerance, Lipid Profile, CRP (nephelometry). MS: ATPIII criteria.

Results: CRP levels ≥ 1 mg/l a) Normoglycemics (NG) 65.2%, impaired fasting glucose (IFG) 6.7%, Impaired glucose tolerance (IGT) 15% and type 2 Diabetes Mellitus (DM2) 13.1%.

Overall MS prevalence 20.4%, F: 23.6%/M: 16.7% ($p<0.001$). MS in NG: 10.5%, IFG: 57.8%, IGT: 25.7%; DM2: 59.5% ($p<0.001$). Obesity (BMI≥30) NG:23.3%, IFG:34.4%, IGT: 35.6% and DM2: 42.6%.

Correlations CRP/Components of MS by sex: M/F statistically significant unadjusted positive correlation with CRP: FG, glucose 2 hs (G2hs), BMI (strongest in F), WC, TG, SBP, DBP ($p<0.003$); significant negative correlation CRP/HDL-C ($p<0.001$). Strongest correlation observed: NG, IGT and DM was with : CRP/BMI ($r=0.29$, 0.39 and 0.30 respectively)and in IFG: CRP with WC and BMI were similar($r=0.38$ and 0.35 respectively).

In subjects with CRP levels≥1 mg/l prevalence of MS increased a) NG OR:1.53 (IC 95% 1.03–2.29), b) IFG OR: 5.55 (IC 95% 2.48–12.40), c) IGT OR: 2.95 (IC 95%1.17–7.49), d) DM2 OR: 3.64 (1.74–7.63). Prevalence of MS in NG was significant after adjusting by fasting glucose/G2hs., in IFG after adjusting by BMI/WC/TG/HDL-C/BP/FG; in IGT after adjusting for all abnormalities including BMI/G2hs, in DM2 only after adjusting by WC. Higher prevalence of MS adjusted by the abnormalities of MS presented in IFG.

CRP levels increased with number (2 or more) abnormalities of MS ($p<0.001$) and degree of glucose tolerance ($p<0.001$).

The prevalence of MS increased 1.68 (IC 95% 1.21–2.35) fold in individuals with CRP≥1.0 mg/l age/sex/BMI/IGT adjusted by logistic regression model.

Conclusion: It appears that CRP levels over≥1.0 mg/l are associated with criteria (ATPIII) of Metabolic Syndrome; obesity and glucose intolerance in a stepwise dependent manner.

0738

Excretion of catecholamines and serum uric acid levels in obese patients with type 2 diabetes mellitus and metabolic syndrome

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Background and Aims: Metabolic syndrome (MS) is associated with increased activity of sympathoadrenal system (SAS). Elevated level of serum uric acid (UA_s) is a sign of MS. Uric acid can be consider an activator of SAS because it has a structural similarity with methylxantines. The aim of the present investigation is to study association between activity of SNS and uricemia, inslin resistance (IR), insulinemia in patients with type 2 diabetes mellitus (DM2).

Materials and Methods: 49 subjects (M/F 27/22, aged 55.9±1.5 yr) with DM2 and signs of MS: abdominal obesity, hypertension, fasting hyperglycemia, cholesterolemia, and 17 age- and BMI- matched non-diabetic controls were studied. In 23 diabetics and 9 controls, content of catecholamines (CA) was measured in fractions of urea collected during 24 hours in periods: I - 8:00–12:00, II - 12:00–16:00, III - 16:00–18:00, IV - 20:00–8:00.

Results: UA_s in diabetic subjects was 326±21 μmol/L (in women) and 361±15 (in men); HOMA-IR was 4,06±0,6 and 4,01±0,5, respectively, with no difference with control ($p>0.05$). UA_s correlated with BMI ($r=0.45$; 0.58), insulin ($r=0.62$; 0.42), HOMA-IR ($r=0.69$; 0.45), $p<0.01$. Positive correlation of CA urinary excretion (CA_u), blood pressure and heart rate ($p<0.05$) indicates that SNS activity may be increased in the patients. Correlation between diabetes duration and CA_u: adrenaline, noradrenaline, dopamine in I period ($r=-0,48$; $-0,43$; $-0,42$) and in III period ($r=0,32$; 0,31; 0,33, resp.) was found, so the patients were divided in 2 groups: A - duration of DM <5 yr (n=12), and B −>5 yr (n=11). Different shapes of day's CA_u profiles in the groups were revealed. In cohort A of diabetics, as well as in control subjects, decreased CA_u in III period (comparing to I period) was found. In contrast, CA_u in B group was growing constantly. These data suggest that patients of group B develop disorder of day's CA excretion rhythm. This may be in parallel with development of DM: level of C-peptide in B cohort was decreased comparing with A (2,62±0,6 vs 5,61±1,1 ng/ml). UA_s in IV period negatively correlated with C-peptide in group A ($r=-0.70$) and in B ($r=-0.36$) $p<0.05$. A strong positive correlation of UA_s with CA_u in IV period ($r=0.72$; 0.62; 0.82, $p<0.05$) in patients of group A, and negative correlation with adrenaline and dopamine excretion in B ($r=-0.61$; -0.80, $p<0.05$) was found. CA_u in diabetic patients exceeded normal rate, this suggested hyperproduction of UA. In group A UA_s was increased in comparison with B (11.2±0.9 vs 5.8±0.5 mmol).

Conclusion: The results demonstrate that relationship between activity of SNS (assessed by urinary excretion of CA) and characteristics of MS (IR, insulinemia, uricemia) in obese patients with type 2 DM was more prominent in subjects with duration of diabetes less then 5 yrs, under conditions of relatively high serum levels of insulin and

uric acid. This may be accounted for hypouricosuric action of insulin and stimulating effect of UA on SNS activity. In patients with DM>5 yrs disorders of daily rhythm of CA excretion were revealed.

0739

Insulin resistance and hypertension

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Background and Aims: The association between insulin resistance (IR), hyperinsulinemia and essential hypertension is controversial. The aim of this study was to examine the IR estimated by homeostasis model assessment (HOMA-IR) in diabetic and non diabetics with and without hypertension.
Materials and Methods: A total of 607 subjects between November 2004 and December 2005 who were referred to a general hospital were included in a cross sectional study. We analyzed data of 150 non-diabetics (75 normal, 75 hypertensive) and 150 diabetic (75 normotensive, 75 hypertensive) age-BMI matched patients. Demographic data, anthropometric measurements were recorded. Smokers and patients on insulin therapy were excluded. Fasting blood samples were collected and FBS, serum creatinine, electrolytes, lipids, insulin (FPI), C-peptide, HbA1c were measured. HOMA-IR was calculated as FBS (mg/dl)×FPI (mU/l)/405 and HOMA-B was calculated as (20×FPI)/[(FBS/18)–3.5)]. Regression analysis was done to determine the association between HOMA-IR and various components of metabolic syndrome.
Results: The age, BMI, waist circumference, fasting insulin, C-peptide, HOMA-IR and HOMA-B values are summarized in table 1. These data show that HOMA-IR and HOMA-B were statistically different in diabetic patients versus non diabetic individuals with and without hypertension ($p<0.001$ for both), however, there was not significant difference between hypertensive versus control groups. The relative contribution of history of DM, HTN, sex, age, BMI, waist circumference, serum lipids, and mean arterial pressure (MAP) was analyzed to predict HOMA-IR by multiple regression analysis. History of DM, HTN, age, waist circumference, MAP, TG, and HDL levels were significantly independent predictor variables ($r=0.46$, $P=0.001$). The best predictors of HOMA-IR were the age and waist circumference in addition to DM.
Conclusion: These results do not support the evidence that insulin resistance and insulinemia are increased in hypertensive patients. IR is associated with the history of DM, age, and central obesity.

Demographic and insulin indice of participants

Group	Age (mean± SD)	BMI (mean± SD)	Waist (mean± SD)	Insulin (mean± SD)	C-Peptide (mean± SD)	HOMA-IR (mean± SD)	HOMA-B (mean± SD)
Normal	55±8	29.89± 4.55	97±9	6.52± 3.15	2.39± 1.1	1.57± 0.81	74.7± 40.96
HTN	56±8	29.65± 4.51	100±11	7.45± 4.13	3.04± 1.38	1.82± 1.01	85.15± 69.03
DM	55±8	29.5± 4.79	98±12	8.62± 6.73*	3.21± 1.75*	3.54± 3.15*	35.09± 28.29*
DM+ HTN	56±7	29.76± 4.94	99±9	6.22± 3.39	2.91± 1.21	2.51± 1.47*	26.17± 18.44*

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0740

Which plays more important role in pathogenesis of metabolic syndrome, the fat accumulation in visceral cavity or in the liver?

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Background and Aims: Metabolic syndrome (MetS) has been highlighted as an important risk for cardiovascular disease. Visceral adiposity (VA) is generally thought to be a prerequisite to the diagnosis of MetS. However, it has been shown that the percent liver fat, but not the visceral fat, is a parameter best correlated with a hepatic insulin resistance and the risk of hepatic steatosis increases exponentially with each addition of a component of the MetS, such as impaired glucose tolerance, hypertension (HT), and dyslipidemia. The aim of this study was to investigate which is more etiologically important for MetS, the fat accumulation in visceral cavity or in the liver.
Subjects and Methods: The study subjects were 962 men (age: mean±SD, 50±8 years) and 495 women (50±8 years) in a cohort of middle-aged apparently healthy adults who took medical examinations including measurement of waist circumference (Wc) at the height of umbilicus, the surrogate measure of visceral adiposity and B-mode ultrasonography of the liver. The Japanese diagnostic criteria for MetS are as follows; Wc≥85 cm (men),≥90 cm (women) plus any two of the following three: BP≥130/85 or under antihypertensive treatment as HT, TG≥150 mg/dl, HDL-C <40 mg/dl or under

lipid lowering agents as dyslipidemia, FBS ≥110 mg/dl or under treatment of diabetes as hyperglycemia. The sex specific prevalence of nonessential components of MetS was calculated by tertile of Wc and Body Mass Index (BMI). Further stratified analysis was made by the presence or absence of fatty liver (FL). The independent impact of FL on the presence of MetS was analysed by the logistic regression model, after adjusting for age.

Results: 1) The prevalence of the MetS was 22.6% for men and 4.4% for women. 2) The aggregation rate of each component of MetS was almost identical when compared with the stratification by BMI and Wc (each $p<0.0001$ for linear trend). 3) The prevalence of FL was 13.7% for men and 2.4% for women. 4) In men, through the three strata by Wc, all of the prevalence of HT, Dyslipidemia (high TG, low HDL-C) were insignificantly higher in the FL group than in the non-FL group, but that of FBS was significantly higher in the FL group of the lowest and middle Wc group (Table 1). In women, a similar tendency was found without a statistical significance. 5) The age-adjusted odds ratios of having MetS associated with FL was 2.04 (95% CI 1.50–2.78) for men and 1.44 (0.60–3.49) for women.

Conclusions: These results suggest but do not confirm that FL is more important than visceral adiposity in generating MetS.

Prevalence of components of the MetS by status of the liver and Wc in men

	Lowest Wc (<82.5 cm)		Middle Wc (82.5–88.4 cm)		Highest Wc (≥88.5 cm)	
	normal (n=126)	FL (n=176)	normal (n=148)	FL (n=169)	normal (n=178)	FL (n=165)
HT	43.8	42.9	53.8	57.4	53.3	59.0
Dyslipidemia	20.5	29.4	39.1	50.7	46.7	56.2
High TG	11.9	17.5	27.8	40.5	36.4	44.4
Low HDL	7.4	11.1	15.4	15.5	21.2	24.7
High FBS	10.2	24.6	14.8	20.9	23.0	25.8

0741

Insulin sensitivity and fasting insulin: association with indicators of cardiovascular disease in healthy subjects. The RISC Study

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Background and Aims: Insulin resistance is believed to underlie the clustering of cardiovascular disease (CVD) risk factors. However, elevated insulin levels per se may be also implicated in the pathogenesis of CVD. Previous studies have not distinguished between insulin level and insulin sensitivity. We studied this question in a European study of healthy subjects.

Methods: In the RISC Study (Relationship between Insulin Sensitivity and Cardiovascular disease), 1538 men and women were recruited in18 European centres. Inclusion criteria were: age 30–60 years, no history of CVD, blood pressure <140/90 mm Hg, serum total cholesterol <7.8 mmol/l, serum triglycerides <4.6 mmol/l, and plasma glucose (fasting/postload glucose) <7.0/11.1 mmol/l. A euglycaemic hyperinsulinaemic (40 $mU \cdot min^{-1} \cdot m^{-2}$) clamp was performed to assess insulin sensitivity (M value). Low insulin sensitivity was defined by values below the first quartile of the population M value. High insulin was defined by values above the upper quartile of fasting plasma insulin. CVD risk factors were assessed using a standardised protocol. Intima media thickness (IMT) was assessed as an indicator of atherosclerosis, and IMT above the upper quartile was considered as high IMT. A resting 12-lead electrocardiogram (ECG) was made to assess subclinical heart disease. ECG abnormality was defined as the presence of Minnesota Codes 1.1 and 1.2, any 4 and 5 or 7.1. Urinary albumin and creatinine concentrations were assessed, and microalbuminuria, defined as albumin to creatinine ratio≥2, was considered a marker of impaired endothelial function. We computed odds ratios for the presence of the metabolic syndrome (NCEP criteria), high IMT, ECG abnormalities or microalbuminuria in subjects with high insulin and subjects with low insulin sensitivity, adjusted for age, gender and body mass index (BMI).

Results: Information on all variables was available for 1089 subjects. High fasting insulin was more strongly associated with the metabolic syndrome and microalbuminuria than low insulin sensitivity, but insulin sensitivity was associated with ECG abnormalities (table).

Conclusion: These results support the hypothesis that other mechanisms besides insulin resistance contribute to the clustering of CVD risk factors in the metabolic syndrome,

but that insulin resistance may be related to sub-clinical heart disease.

Odds Ratios (95% CI) of low insulin sensitivity and high fasting insulin for indicators of CVD

	Prevalence	Low Insulin Sensitivity	High Fasting Insulin
NCEP Metabolic Syndrome	7%	2.9 (1.6–5.1)	5.0 (2.7–9.1)
IMT in upper quartile	25%	1.0 (0.6–1.5)	1.4 (0.9–2.3)
ECG abnormality	10%	3.2 (1.5–7.0)	1.9 (0.8–4.3)
Micro-albuminuria	3%	2.2 (1.5–3.4)	3.3 (2.1–5.1)

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Relationship between insulin-resistance, metabolic syndrome and altered glucose tolerance. The GENFIEV Study

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Background and Aims: Insulin-resistance (IR) is a characteristic finding in prediabetic individuals, predicts the development of type 2 diabetes and is the defect underlying metabolic syndrome (MS), but the relationship between IR and the traits of the MS is not yet well defined. Aim of this study was to evaluate the association between IR and MS among subjects with abnormal glucose regulation (AGR).
Materials and Methods: From the database of GENFIEV (GENetics PHYsiopathology and EVolution of type 2 Diabetes), an Italian nationwide study designed to recruit individuals with AGR, we studied 1017 subjects (589 men/ 428 women, age 49±11, BMI 29,2±5,3 Kg/m2). In all individuals we performed an OGTT with measurement of glycemia, c-peptide and fasting insulin and evaluated anthropometric and biochemical parameters to define MS by NCEP-ATP III criteria.
Results: The most represented MS trait was abdominal obesity (59%), followed by hypertension (53%), low HDL (33%), high triglycerides (33%) and hyperglycemia (27%). The prevalence of MS in the population study was 35% (31% in men and 40% in women); 21,5% in subjects with normal glucose tolerance (NGT), 43,5% in those with impaired fasting glucose (IFG), 33,3% in those with impaired glucose tolerance (IGT), 75% in IFG+IGT subjects, 63% in newly diagnosed diabetic patients. The prevalence of IR (HOMA>75° percentile of controls) was 61% in subjects with MS (Chi-Square: p<0,0001) and increased according to the traits number: 52%, 69% and 91% in presence of 3, 4 or 5 traits, respectively. Among subjects with ARG, 42% was IR (Chi-Square: p<0,0001) and those with IFG+IGT showed an higher frequency of IR than those with the isolated defect (28% vs 10% in IFG and 13% in IGT; Chi-Square: p<0,0001). The 70% of subjects with MS was identified by 3 traits; the association of obesity, hypertriglyceridemia, low HDL and hypertension was present in more than 50% of the cases. The combinations of 3 traits including hyperglycemia and/or obesity were those with the highest predictive power to identify subjects with MS (PPV 50–100%).
Conclusion: This study showed that in subjects with abnormal glucose regulation the prevalence of MS is high (44%) and is generally associated to IR, and some combinations of traits of the MS may significantly contribute to identify subjects with IR.
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PS 56

Metabolic syndrome and lipid lowering in type 2 diabetes

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Metabolic syndrome in young adults with type 1 diabetes mellitus

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Background and Aims: Metabolic syndrome (MS) is a risk factor for vascular complications. The prevalence of MS in type 1 diabetic patients is unknown. The aim of the study was to estimate the prevalence of metabolic syndrome diagnosed according to the definitions of International Diabetes Federation (IDF) in own modification, in young adults with type 1 diabetes.
Materials and Methods: 142 patients with type 1 diabetes mellitus (62 females and 80 males) aged from 18 to 35 years (mean: 23.0±4,0 years) were included into the study. The diabetes duration ranged from 4 to 26 years (mean: 14.1±5.4 years). The height, weight, waist circum-

ference and blood pressure were measured. HbA1c (HPLC method) and plasma lipids (enzymatic method) concentrations were examined. Because in type 1 diabetics hyperglycemia is mainly caused by insulin deficiency, the criterion of hyperglycemia was replaced with the criterion of insulin resistance. Euglycemic-hyperinsulinemic clamp was performed to establish insulin resistance. In patients with glucose disposal rate below 6.5 mg/kg/min insulin resistance was diagnosed. Body mass index (BMI) and daily dose of insulin were calculated.

Results:The presence of MS was found in 15 patients (10.6%). Abdominal obesity was observed in 27 patients (19.0%). Lowered plasma HDL-cholesterol levels were noted in 12 individuals from the study group (8.5%), and hypertriglicerydemia - in 12 (8.5%) of them. Blood pressure exceeding 130/85 mmHg was observed in 45 patients (31.7%). Glucose disposal rate ranged from 2.0 to 15.5 mg/kg/min. Insulin resistance was diagnosed in 62 individuals (43,.7%). In the study group mean BMI was 24.5±4.1 kg/m^2, HbA1c − 7,6±1.2%, daily dose of insulin − 0.86±0.2 IU/kg and 33.5±8.5 IU/m^2. Logistic regression analysis showed association between prevalence of MS and patients age (OR: 1.27; p=0.002) and BMI (OR: 1.9, p<0.001). No statistically significant relationship was found between MS prevalence and HbA1c, diabetes duration or sex. In patients with MS daily dose of insulin calculated per unit of body surface area was higher than among patients without MS (41.7 vs. 32.5 IU/m^2, p<0.0001).

Conclusion: Metabolic syndrome, as defined by IDF criteria in own modification, is not a very frequent finding in young type 1 diabetic subjects. The elder age and higher BMI were associated with prevalence of MS.

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0744

Insulin secretion and insulin sensitivity in subjects with impaired glucose regulation

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Background and Aims: Substantial progress in understanding the underlying defects in diabetes mellitus has led to an etiological classification of the disorder. However, the same has not yet been possible for the prediabetic stages of impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) collectively termed as Impaired Glucose Regulation (IGR) by WHO study Group. There are still incompatibilities between the clinical classifications of IFG and/or IGT proposed by WHO Study Group and ADA Expert Committee and, in particular, the phenotype of combined IFG and IGT has not been adequately addressed. Understanding of the pathophysiology is a prerequisite for further characterization of these conditions. The present study was undertaken to explore the pancreatic B cell function and insulin sensitivity of the clinical types of IGR in a Bangladeshi population.

Materials and Methods: Following ADA 1997 and WHO 1999 guidelines 13 IFG and 112 IGT subjects, along with 39 healthy controls, aged>20 years, were selected purposively from the out-patient department of BIRDEM (the tertiary hospital of Diabetic Association of Bangladesh). During analysis, we also categorized IGT subjects into 91 isolated IGT (I-IGT) and 21 combined IFG-IGT subjects. Fasting and 2 h-plasma glucose and insulin (by chemiluminescent immunoassay) were measured. Insulin secretory capacity (%B) and insulin sensitivity (%S) were assessed by homeostasis model assessment (HOMA) using HOMA-CIGMA software.

Results: There was significantly higher fasting and 2 h-plasma insulin in IGT subjects compared to Control. HOMA %B was significantly decreased in IFG compared to IGT and Control [Median (range); IFG, 60 (34–164); IGT, 83 (36–235); Control, 90 (41–251)], but there was no difference between IGT and Control. There was significantly compromised HOMA %S in IGT compared to Control [IGT, 88 (22–266); Control, 121 (37–371)]. When IGT subjects were categorized into I-IGT and IFG-IGT, it was found that plasma insulin was raised in I-IGT and IFG-IGT compared to control. HOMA %B did not differ significantly in I-IGT [92 (36–235)] compared to Control, but it was significantly higher compared to IFG and IFG-IGT [75 (36–138)]. The IFG-IGT had significantly lower value compared to Control. HOMA %S was significantly compromised in both categories of IGT [I-IGT, 91 (22–266); IFG-IGT, 71 (31–165)]. HOMA %S was decreased but just outside the range of statistical significance in IFG compared to Control (p=0.084) and in IFG-IGT to I-IGT (p=0.069).

Conclusion: The pathophysiological mechanisms differ in IFG (primary defect of B-cell dysfunction with a tendency to insulin resistance) and isolated IGT (primarily an insulin resistant condition). The pathophysiology in the clinical entity of combined IFG-IGT (both B-cell dysfunction and insulin resistance) seems to characterise it as a different clinical entity and, thus, it may not be included in IGT as done by ADA and WHO.

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Evaluation of islet beta cell function in newly diagnosed type 2 diabetic patients

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Background and Aims: Clinical studies have shown that short-term intensive insulin treatment in newly diagnosed type 2 diabetic patients yielded prolonged remission. To investigate the effects of different treatments (oral anti-hyperglycemic agents, multiple daily injection or continuous subcutaneous insulin infusion) on β cell function and prognosis of newly diagnosed type 2 diabetes mellitus, we conducted this multicenter, perspective clinical trial. Here we evaluated the baseline function of islet β cell in recruited subjects and compared the different indexes assessing insulin secretion capacity.
Materials and Methods: 352 newly diagnosed type 2 diabetic patients and 48 subjects with normal glucose tolerance (NGT) underwent intravenous glucose tolerance tests (IVGTT). Serum samples were obtained before and 1, 2, 4, 6, and 10 min after injection for insulin determination. The acute insulin response (AIR) and the area under the curve of insulin (AUC) were used to evaluate the insulin secretion. HOMA B was another indicator of insulin secretion. HOMA IR was used to assess insulin resistance. To represent another profile of β cell dysfunction, the fasting plasma proinsulin level was measured and the ratio of fasting proinsulin to fasting insulin (PI/I), an index representative of reduced conversion of proinsulin to insulin, was calculated.
Results: Both AUC and AIR during IVGTT were evidently reduced in type 2 diabetic patients compared with NGT subjects. In particular, as FPG was above 7.0 mmol/l, AIR had been evidently reduced significantly. And AIR subsequently disappeared when FPG was above 9.0 mmol/l. After adjusted by HOMA IR, HOMA B of the type 2 diabetic patients was reduced compared with the NGT subjects (about 30% of the normal level). Both fasting proinsulin concentration and the ratio of PI/I were significantly higher in T2DM than that in NGT subjects (22.6±14.7 pmol/l vs. 11.5±7.1 pmol/l, $p<0.001$; 30.1±20.5% vs. 12.1±9.6%, $P<0.001$, respectively). In diabetic population, AIR, AUC and HOMA B were significantly negatively correlated with FPG ($P<0.001$), and PI/I positively correlated with FPG ($P<0.001$).
Conclusion: The dysfunction of islet β cell in newly diagnosed type 2 diabetic patients was mainly represented by the disappearance of AIR and the evident decline of AUC and HOMA B, companied with qualitative decreasing of insulin secretion.
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Effects of co-administered ezetimibe and fenofibrate in mixed dyslipidaemic patients with metabolic syndrome

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Background and Aims: Metabolic syndrome (METS) is a clustering of atherosclerotic CHD risk factors including abdominal obesity, decreased HDL-C, and increased fasting plasma glucose, triglycerides (TG), and blood pressure. According to NCEP ATP III, the dyslipidemia defined by the METS is a secondary treatment target after LDL-C. This post-hoc analysis evaluated the treatment response of an LDL-C lowering therapy, ezetimibe (EZE), co-administered with a TG-lowering/HDL-C raising therapy, fenofibrate (FENO) in patients with both mixed dyslipidemia and metabolic syndrome.
Materials and Methods: Data were derived from a cohort of 625 patients with mixed dyslipidemia that were randomized in a 1:3:3:3 ratio to 1 of 4 treatments for 12 weeks: placebo; EZE 10 mg; FENO 160 mg; or EZE 10 mg+FENO 160 mg. Patients were 18–75 years old, with LDL-C 3.36–5.69 mmol/L [2.59–4.65 mmol/L for patients with type 2 diabetes], TG 2.26–5.65 mmol/L, with no history of CHD or CHD risk>20%, or other CHD risk equivalent disease (except for type 2 diabetes). Metabolic syndrome status was determined in 622 evaluable patients by NCEP ATP III criteria.
Results: At baseline, patients with metabolic syndrome were more obese, had higher TG, and lower HDL-C, and tended to have LDL size pattern B compared to those without metabolic syndrome. Treatment with EZE and

FENO alone or when co-administered produced comparable improvements in lipid parameters irrespective of the presence of metabolic syndrome (Table). Treatment with EZE+FENO and FENO also shifted more patients away from the smaller, more dense LDL size pattern B after 12 weeks, which was similar between the subgroups.

Conclusions: In this post-hoc analysis, co-administration of EZE+FENO had similar lipid profile benefits in patients with mixed dyslipidemia and with or without the presence of metabolic syndrome.

	LS Mean Percent Change from Baseline			LDL Size Pattern B, n (%)	
Treatment	LDL-C	TG*	HDL-C	Baseline	Week 12
Metabolic Syndrome (n=450)					
Placebo	1.1	−6.9	3.3	34 (76)	30 (67)
EZE	−14.0	−9.8	3.0	96 (80)	74 (62)
FENO	−3.0	−44.2	20.4	93 (79)	24 (20)
EZE+FENO	−20.4	−45.1	19.0	90 (77)	21 (18)
No Metabolic Syndrome (n=172)					
Placebo	−5.1	−13.7	2.0	6 (46)	6 (46)
EZE	−10.5	−20.7	7.2	32 (73)	24 (55)
FENO	−11.3	−42.0	15.0	34 (67)	10 (20)
EZE+FENO	−21.4	−41.4	18.8	33 (69)	8 (17)

*median % change; With ANCOVA, treatment by subgroup interaction term was not significant for lipid parameters, except LDL-C (p=0.02).

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Effects of ezetimibe/simvastatin on atherogenic ratios ApoB/ApoA1 and LDL-C/HDL-C

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Background: The ratios of low density lipoprotein-cholesterol to high density lipoprotein-cholesterol (LDL-C/HDL-C) and apolipoprotein B to apolipoprotein A-1 (Apo B/Apo A-1) have been shown to be good predictors of cardiovascular risk and plaque progression. This analysis compared the effects of ezetimibe (EZE), simvastatin (SIM) and ezetimibe/simvastatin (EZE/SIM) on Apo B/Apo A-1 and LDL-C/HDL-C in patients with primary hypercholesterolemia.

Methods: This *post hoc* analysis was performed on data collected from 3 similar randomized, placebo (Pbo)-controlled, double-blind studies. After a 6- to 8-wk washout and 4-wk diet/Pbo run in, 3083 hypercholesterolemic patients (LDL-C: 145–250 mg/dL; TG≤350 mg/dL) were randomized equally to placebo (Pbo), EZE 10 mg, EZE/SIM (10/10, 10/20, 10/40, 10/80 mg), or SIM (10, 20, 40, 80 mg) for 12 weeks. Mean percent change from baseline in Apo B/Apo A-1 and LDL-C/HDL-C were examined across individual and pooled treatment groups.

Results: Relative to Pbo, EZE alone significantly reduced Apo B/Apo A-1 and LDL-C/HDL-C ($p<0.001$ for both comparisons; see Table). When pooled across all doses, SIM and EZE/SIM produced greater reductions in Apo B/Apo A-1 and LDL-C/HDL-C compared to EZE alone ($p<0.001$ for all comparisons). The Apo B/Apo A-1 and LDL-C/HDL-C lowering efficacy of EZE/SIM was greater than that observed with SIM alone when pooled across doses and at each of the corresponding dose comparisons ($p<0.001$ for all comparisons). The enhanced Apo B/Apo A-1 and LDL-C/HDL-C lowering effects of EZE/SIM versus SIM were consistent across subgroups based on age (<65 vs≥65 years), gender, body mass index (<30 vs≥30 kg/m^2), and patient history of coronary heart disease, diabetes, and metabolic syndrome status.

Conclusions: Compared to Pbo, EZE alone produced significantly greater improvements in lipoprotein ratios in patients with primary hypercholesterolemia. Treatment with EZE/SIM significantly reduced these ratios relative to EZE and SIM alone.

	Mean % change from baseline±SE			
	Pbo	EZE 10	Pooled SIM†	Pooled EZE/SIM†
Apo B / Apo A-1	0.31±0.88	−16.31±0.71*	−33.41±0.43	−43.92±0.43**
(N)	(296)	(287)	(1177)	(1167)
LDL-C / HDL-C	−0.38±0.91	−22.59±0.73*	−41.55±0.45	−55.47±0.45**
(N)	(307)	(297)	(1218)	(1226)

†Treatment (LS) means are based on the ANOVA model with term for treatments (10).
*p-value <0.001 versus Pbo.
** p-value <0.001 versus pooled SIM.

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Efficacy of ezetimibe/simvastatin 10/20 mg and 10/40 mg compared to atorvastatin 20 mg in type 2 diabetic patients

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Background and Aims: This multi-center, randomized, double-blind study evaluated the efficacy and safety of switching from atorvastatin 10 mg to ezetimibe/simvastatin (EZE/SIM) 10/20, EZE/SIM 10/40 mg or doubling the dose of atorvastatin (ATV) to 20 mg in patients with Type II diabetes (T2DM).

Materials and Methods: Eligible patients had $HbA_{1C}\leq$ 10%, were aged≥18 yrs and were taking ATV 10 mg for ≥6 wks before study entry. Following 4 wks of open-label ATV 10 mg/day, patients were randomized 1:1:1 to EZE/SIM 10/20 mg/day (n=220), EZE/SIM 10/40 mg/day (n=222), or ATV 20 mg/day (n=219) for 6 wks. The primary endpoint was mean % reduction in low-density lipoprotein cholesterol (LDL-C).

Results: Treatment with EZE/SIM 10/20 and 10/40 mg produced significant reductions in LDL-C, total cholesterol (TC), non-high-density lipoprotein cholesterol (non-HDL-C), and apolipoprotein B (Apo B) relative to ATV 20 mg (Table). There were no significant differences in HDL-C and triglycerides (TG) between EZE/SIM and ATV. Significantly more patients achieved LDL-C goal <2.6 mmol/L with EZE/SIM 10/20 and 10/40 mg than with ATV (91%, 87%, and 70%, respectively; p≤0.001). Both EZE/SIM doses were generally well tolerated, with an overall safety profile similar to ATV. There were no differences in the incidences of liver transaminase or creatine kinase elevations between groups.

Conclusions: EZE/SIM 10/20 and 10/40 mg, dual inhibition treatment strategies targeting both cholesterol synthesis and absorption, were well tolerated and provided greater lipid-altering efficacy than doubling the dose of ATV in T2DM patients.

Parameter†	EZE/SIM 10/20 mg	EZE/SIM 10/40 mg	ATV 20 mg
LDL-C (%)	−26.2*	−30.1*	−8.5
TC (%)	−14.2*	−16.8*	−5.5
HDL-C (%)	2.4**	1.3 **	1.6
TG (%)††	−9.7 **	−8.4 **	−5.5
Non-HDL-C (%)	−20.9*	−23.8*	−7.4
Apo B (%)	−14.9*	−19.5*	−6.7

†Least-squares mean percent change from baseline to endpoint
††Median percent change from baseline to endpoint
*p≤0.001 vs. ATV 20 mg
**not significant, p≥0.117 vs. ATV 20 mg

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Elevated serum retinol binding protein 4 levels with triglycerides in obesity and type 2 diabetes mellitus

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Background and Aims: Retinol binding protein 4 (RBP4) is a cytokine that is mainly expressed in hepatocytes. Recent studies in animal models have shown that RBP4 is also expressed in adipocytes and elevated RBP4 levels in the plasma may contribute to the pathogenesis of Type 2 diabetes mellitus (T_2DM). However, the pathophysiological roles of RBP4 in humans remain largely unknown. In this study, we investigated whether the human serum RBP4 levels are altered in patients with obesity and T_2DM and the relationship between RBP4 and lipid metabolism.

Materials and Methods: Enrolled subjects (n=131, age 44.7±11.6 years old) were divided into 4 groups: normal control (NC) (n=31), normal glucose regulation with overweight or obesity (OW/OB-NGR) (n=31), type 2 diabetes mellitus with normal weight (NW-T_2DM) (n=33), and type 2 diabetes mellitus with overweight or obesity (OW/OB-T_2DM) (n=36). Serum RBP4 and insulin were measured by radioimmunology.

Results: After age and sex adjusted, serum RBP4 levels in OW/OB-NGR and OW/OB- T_2DM were significantly elevated compared to the NC group (29.85±6.60*vs* 25.47±6.84 μg/ml, 30. 86±6.11*vs* 25.47±6.84 μg/ml, $P<0.01$, $P<0.01$). However, there is no significant difference between NC and NW-T_2DM (29.85±6.60*vs* 28.35±5.42 μg/ml). The serum RBP4 level in OW/OB-T_2DM is significantly higher than that of NW-T_2DM (30. 86±6.11*vs* 28.35±5.42 μg/ml $P<0.05$). Multiple linear regression analyses reveal that WHR ($r^2=0.166$, $P<0.001$), triglyceride (TG) ($r^2=0.188$, $P<0.001$) and age ($r^2=0.205$, $P<0.001$) are independent variables for RBP4. Because there was no correlation between the fasting plasma glucose and RBP4 levels, total of 131 subjects were re-divided into 4 groups: normal TG (TG <1.7 mmol/L) with normal WHR (WHR≤0.90 in male, WHR≤0.85 in female) group (n=39), high TG with normal WHR group (n=11), normal TG with large WHR group (n=43) and high TG with large WHR group (n=38). After age, sex and WHR adjusted, serum RBP4 levels in the high TG group were significantly higher than that of the normal or large WHR groups with normal TG ($P<0.05$). However, there was no significant difference in the RBP4 levels between the normal WHR group and large WHR group ($P<0.05$).

Conclusion: Serum RBP4 levels are significantly increased in obese Chinese subjects. The elevated RBP4 levels are

correlated with visceral obesity and, more importantly, lipid metabolism.

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0750

ACE inhibition and insulin sensitivity in obese insulin resistant subjects: a double-blind placebo-controlled randomized trial to examine underlying mechanisms

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Background and Aims: Recent clinical trials have demonstrated that long-term angiotensin-converting enzyme (ACE) inhibitor treatment reduces the incidence of type 2 diabetes. This study was designed to investigate the effect of short-term ACE inhibitor treatment on insulin sensitivity and to examine for the first time possible underlying metabolic and hemodynamic effects in obese insulin resistant subjects.
Materials and Methods: A double-blind placebo-controlled randomized trial was performed in 18 obese insulin resistant men (age, 53±2 yr; BMI, 32.6±0.8 kg/m^2; HOMA-IR, 5.6±0.5; SBP, 140.8±3.2 mmHg; DBP, 88.8± 1.6 mmHg), free of any medication, to examine the effects of 2-week ACE inhibitor treatment (ramipril, 5 mg/d) on insulin sensitivity, forearm blood flow, substrate fluxes across the forearm, whole-body substrate oxidation, and intramuscular triacylglycerol (IMTG) content.
Results: Ramipril treatment significantly decreased ACE activity compared with placebo (−22.0±1.7 *vs.* 0.2±1.1 U/l, respectively, $P<0.001$), resulting in a significantly reduced blood pressure (SBP, −10.8±2.1 *vs.*−2.7±2.0 mmHg, respectively, $P=0.01$; DBP, −10.1±1.3 *vs.*−4.2±2.1 mmHg, respectively, $P=0.03$). Ramipril treatment had no significant effect on whole-body insulin-mediated glucose disposal (before: 17.9±2.0 *vs.* after: 19.1±2.4 μmol·kg bw^{-1}·min^{-1}, $P=0.44$), insulin-mediated glucose uptake in skeletal muscle (before: 1.82±0.39 *vs.* after: 1.92±0.29 μmol·100 ml forearm tissue^{-1}·min^{-1}, $P=0.81$), and IMTG content (before: 45.4±18.8 *vs.* after: 48.8±27.5 μmol·mg dry muscle^{-1}, $P=0.92$). Furthermore, the increase in carbohydrate oxidation ($P<0.001$) and forearm blood flow ($P<0.01$), and the decrease in fat oxidation ($P<0.001$) during insulin stimulation were not significantly different between treatments.
Conclusion: Short-term ramipril treatment adequately reduced ACE activity and blood pressure, but had no significant effects on insulin sensitivity, forearm blood flow, substrate fluxes across the forearm, whole-body substrate oxidation, and IMTG content in obese insulin resistant subjects. The present data suggest that the reduced incidence of type 2 diabetes after long-term ACE inhibition can be explained by long-term effects of these agents that affect insulin sensitivity or insulin secretion.

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BGP-15, a new insulin sensitizer targeting mitochondria

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Background and Aims: Validation of the experimental and clinical insulin sensitizing activity of BGP-15, a hydroxylamine drivative.
Materials and Methods: Insulin sensitizing effect of BGP-15 was tested both in animals and in humans. Hypercholesterolemic, insulin resistant rabbits and Goto-Kakizaki diabetic rats were used to demonstrate experimental insulin sensitizing activity. In humans, clinical efficacy was evaluated in 42 insulin resistant patients selected upon HOMA IR, waist-to-hip ratio, and BMI. The insulin sensitizing efficacy was evaluated by hyperinsulinemic euglycemic clamp and by iv glucose tolerance test. The effect of treatment on expression of heat shock proteins (HSP72, HSP90) and constitutive nitric oxide synthases (eNOS, nNOS) was evaluated by Western blotting both in cultured endothelial cells and in tissues of Goto-Kakizaki rats. Mitochondrial mass was evaluated by MitoTracker staining in cell cultures and by the amount of COX IV protein in animal tissues. Nitric oxide production was analyzed by DAF staining and by isotope labelled arginine conversion assay.
Results: BGP-15 increased glucose utilization by 237 and 175%, respectively at dose of 10 mg/kg, p.o in hypercholesterolemic insulin resistant rabbits and Goto-Kakizaki diabetic rats. The 28-day treatment with 200 and 400 mg daily oral doses of BGP-15 increased whole body glucose utilization (M value) by 1,6 and 1,75 mg/kg/min, respectively as compared to the placebo group (ANOVA, p=0,032).

BGP-15 treatment increased the levels of heat shock proteins and restored the expression and function of constitutive nitric oxide synthases both in tissues of Goto-Kakizaki rats and in endothelial cells exposed to hyperglycemia. Restored cNOS activity was accompanied with improved mitochondrial function and increased mitochondrial mass both in cultured endothelial cells and in tissues of diabetic rats. In endothelial cells, BGP-15 alleviated hyperglycaemia-induced increased mitochondrial membrane potential and protected against mitochondrial structural damage. The mitochondrial effect of BGP-15 was greatly reduced when the NO-cGMP pathway was inhibited. BGP-15 did not show any PPARγ agonist activity.

Conclusion: Results show that BGP-15 exerts its insulin sensitizing effect by a novel mechanism that is linked with stimulation of the HSP/cNOS systems and is mediated by multiple downstream pathways including primarily mitochondrial, cellular and NO-mediated neuronal effects. Based on our data BGP-15 represents a novel promising insulin sensitizer drug candidate that affects causal mechanisms of insulin resistance.

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Sex difference in the effect of pioglitazone on anthropometric and metabolic parameters in type 2 diabetic patients

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Background and Aims: The purpose of the present study was to evaluate and explain the sex difference of the clinical effect of pioglitazone on arthropometric and metabolic parameters in type 2 diabetes patients.

Materials and Methods: A total 93 type 2 diabetes patients (men, n=47; women, n=46 ; mean age, men : 48.5±10.0, women 51.5±11.5 years) were studied before and after 3 months, 6 months, and 12 months of pioglitazone treatment. Anthropometric and metabolic parameters were measured.

Results: In both men and women, fasting plasma glucose (FPG) and glycosylated hemoglobin (HbA1c) decreased after 6 months of pioglitazone treatment (P<0.01). The decreases in FPG and HbA1c levels after pioglitazone treatment relative to the baseline were more prominent in women than in men (FPG -11.9 vs.−33.3±41.9 mg/dL, P<0.01; HbA1c,−0.6±1.1 vs. −1.0±1.2%, P=0.062). The decrease in the homeostasis model assessment of insulin resistance(HOMA-IR) index tended to more prominent in women than in men (−0.7±1.6 vs. −1.4±1.3, P=0.09). Body weight and body mass index (BMI) significantly increased after 6 months of pioglitazone treatment only in women. The increases of body weight and BMI was prominent in women than in men (body weight, 0.5±2.4 vs. 2.6±2.1 kg, P<0.01; BMI, 0.2±0.8 vs. 1.1±0.9 kg/m^2). Serum leptin level were increased after 6 months of pioglitazone treatment only in female patient population (14.0±8.0 vs. 17.2±10.1 μg/mL, P<0.01), and serum high sensitivity C reactive protein (hsCRP) level were decreased only in women (2.2±3.6 vs. 0.8±0.7 mg/dL, P<0.05). Serum high density lipoprotein (HDL) cholesterol increased after pioglitazone treatment in both men and women (P<0.01) and, on the other hand, serum triglyceride decreased only in female patient populations (225.5±226.3 vs. 148.3±89.9 mg/dL, P<0.05)

Conclusions: Pioglitazone acts not only on lowering glucose but also on other metabolisms, anti-inflammatory effect and improvement of lipid metabolism, more effectively in women than in men.

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Life style interventions in metabolic syndrome and obesity

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Self reported physical activity after a pregnancy complicated with gestational diabetes (GDM) is associated with less features of the metabolic syndrome

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Background and Aim: We have previously shown a markedly increased prevalence of diabetes and the metabolic syndrome in women with previous GDM. Physical activity is known to have a positive impact on both glucose tolerance and the other components of the metabolic syndrome. The aim of the present study was to examine the level of physical activity and other lifestyle habits among women with prior gestational diabetes mellitus compared with a control group.

Material and Methods: Three-hundred-eighthy-four women of Nordic Caucasian origin diagnosed with gestational diabetes (93% diet treated) in the years 1978–1996, examined at the centre for diabetes and pregnancy, Rigshopitalet, Copenhagen University Hospital at a median of 10 (range 4–23) years after index pregnancy. All women were during pregnancy advised to continue a healthy lifestyle after pregnancy. An age-matched control group of 950 women from a population based cohort study (Inter99) was included. Women with prior GDM not reporting diabetes at follow-up underwent a 2-hour 75 g OGTT (WHO1999), the components of the metabolic syndrome were investigated and the participants completed a self-administered questionnaire regarding physical activity and other lifestyle habits. Physical activity was evaluated by self-estimated physical activity at work and leisure time according to Saltin 1968: Physical inactive: predominantly sedentary at work and leisure time. Light physical activity: Minor activity at work and/or leisure time. Physical activity: At least moderate activity at work and/or leisure time.

Results:

	Prior - GDM			**Control**		
	Physical active	Minor or inactive	*p*	Physical active	Minor or inactive	*p*
n	59 (15%)	325 (85%)	126 (13%)	824 (87%)	NS (GDM vs.control)	
Age, years	40.5 (6.5)	43.2 (7.3)	0.010	44.9 (8.1)	45.1 (8.0)	NS
BMI, kg/m^2	25.3 (5.3)	28.1 (6.5)	0.013	24.3 (5.0)	24.6 (5.2)	NS
Obesity (BMI≥ 30 kg/m^2)	20%	39%	0.006	18%	17%	NS
Diabetes	32%	39%	NS	1%	4%	NS
Metabolic syndrome (WHO)	20%	41%	0.009	10%	14%	NS
Dyslipidaemia	17%	36%	0.017	15%	17%	NS
Hypertension	15%	32%	0.015	27%	31%	NS
Insulin resistance	34%	60%	0.002	18%	26%	NS
Abdominal obesity	29%	54%	0.004	27%	27%	NS
Glucose intolerance or diabetes	54%	68%	NS	9%	19%	0.007
N alcoholic beverages> 14/week	2%	8%	NS	11%	13%	NS
Current smoker	34%	41%	NS	33%	38%	NS

Data presented as percentages or median (SD)

Conclusion: The percentage of physically active women with prior GDM was low and did not differ from the background population. Physically active women with previous GDM had a more favourable health profile regarding the following components of the meatbolic syndrome: dyslipidaemia, hypertension, insulin resistance and abdominal obesity compared to the minor or inactive women. Although our data are cross-sectional they support the notion that women with a history of GDM should be advised to be physically active to improve their overall health profile and reduce their risk of cardiovascular disease.
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0754

The role of lifestyle modification in preventing type 2 diabetes in subjects with impaired glucose homeostasis

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Background and Aims: Leptin is proposed to be involved in regulation of body weight. Only little information is available how lifestyle modification influences on leptin concentrations in individuals with impaired glucose tolerance. The aim of our study was to assess the efficacy of lifestyle modification including altered diet composition and physical activity in preventing diabetes mellitus type 2 (DM 2) and their influence on serum leptin levels in individuals with impaired glucose homeostasis.
Materials and Methods: The study included 327 patients (69m258f) 25–65 y.o. with impaired glucose tolerance/ impaired fasting glucose (IGT/IFG) and newly diagnosed DM 2. Patients were divided into 2 groups matched by sex, age, weight, body mass index (BMI), waist-to-hip ratio (WHR). Research group included 183 patients (33m150f) who received and carried out individual recommendations of a balanced diet and physical activity. Control group included 144 patients (36m108f) who made no lifestyle modifications. The study lasted for 48 weeks. We measured fasting plasma glucose (FPG), 2-hour plasma glucose concentrations (2-h PG) following a 75-g oral glucose tolerance test and related to fasting leptin (FL) concentrations by sensitive ELISA. Comparisons among groups were performed using ANOVA/MANOVA.
Results: Patients of the research group demonstrated reduction of body weight (−4.6±5.2 kg), BMI (−2.3± 3.1 kg/m^2) and WHR (−0.02±0.025) ($p<0.01$ for all). They had positive dynamics of FPG and 2-h PG concentrations

also (−0.5±0.8 and −0.7±0.9 mmol/l respectively; p<0.001). Persons of the control group had significant increase in weight, BMI and WHR as well as FPG and 2-h PG concentrations elevation (p<0.05). The main novel finding was that median serum leptin in the research group decreased by 23.9% (from 34.7 to 26.4 ng/ml, p<0.01) and increased in the control group by 27.6% (from 37.9 to 44.7 ng/ml, p<0.01). Among subjects with IGT from the research group, glucose levels normalized in 49.3% (p<0.001) and serum leptin levels decreased by −8.5 ng/ml, i.e. 26.9% (p<0.01) from initial. In the control group glucose levels normalized in 4.5% (p<0.01) persons with IGT at baseline and combined with FL concentration increase by 7.9 ng/ml (22.9%, p<0.01). By the end of the study 12% of non-diabetic obese subjects of the control group have developed DM 2, 28% subjects - IFG and 48% - IGT, while in the research group there were no changes in glucose concentrations among subjects with normal glucose tolerance (p<0.001). Among patients of the research group there was a reduction of DM 2 by 11.9% and an increase in the control group by 35.1% but FL in the research group did not change significantly.

Conclusion: Thereby, lifestyle modifications lead to reduction not only fasting plasma glucose, 2-hour plasma glucose concentrations but also fasting leptin concentrations in individuals with impaired glucose tolerance.

0755

A lifestyle intervention improves the incretin response during an OGTT

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Background and Aims: The incretin hormones glucose-dependent-insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1) postprandially enhance the glucose-induced insulin response. GLP-1 also plays an important role in controlling appetite, energy intake and satiety. Life style intervention trials with reduced caloric intake and increased physical activity resulted in an improvement of glucose tolerance. Whether incretin secretion is affected by lifestyle intervention is not yet clear. The aim of this study was to investigate the effect of changes in life style on GLP-1 and GIP secretion.

Materials and Methods: 47 (28f/19m) prediabetic persons (age 47±1.6 years, BMI 30.1±0.9 kg/m^2, 35 NGT, 12 IGT) underwent oral glucose tolerance tests (OGTT) before (T0) and 9 months (T1) after life style intervention (calorie restricted diet and regular physical activity). GLP-1 and GIP concentrations (0; 30; 120 min of the OGTT) were analysed with regard to changes of glucose tolerance, insulin secretion and insulin sensitivity estimated by validated indices from the OGTT.

Results: After 9 months of life style intervention GLP-1 concentrations in the OGTT (AUC GLP-1) were significantly higher than in the basal OGTT (T0: AUC GLP-1 3291±262 pmol/l/min, T1: AUC GLP-1 4171±207 pmol/l/min; p=0.006). Furthermore, the increase in total GLP-1 secretion (AUC GLP-1) was positively correlated with a rise in insulin secretion relative to insulin sensitivity (disposition index) (r=0.32, p=0.03) as well as an improvement in glucose tolerance (r=−0.43, p=0.003). Also, a positive correlation was found between the increase in AUC GLP-1 and mean fibre content in the diet (r=0.38, p=0.01). In contrast to GLP-1 no significant changes in total GIP secretion after life style intervention were observed (T0: AUC GIP 7917±403 pmol/l/min, T1: AUC GIP 8840±395 pmol/l/min, p=0.10).

Conclusion: Life style intervention results in an increased GLP-1 response in the OGTT accompanied by an increase in insulin secretion relative to insulin sensitivity and an improvement in glucose tolerance. Moreover, higher fibre intake in the diet correlated with a stronger rise in GLP-1 secretion. The underlying mechanisms are not yet completely understood. Stimulation of gastric receptors, vagal afferent outputs and neural mechanisms might be involved.

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0756

Impact of a school intervention in physical activity or diet on insulin resistance, body mass index, and arterial pressure in adolescents

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Background and Aims: Type 2 diabetes mellitus has increased in adults and adolescents due to obesity, physical inactivity and inadequate food intake. Successful school interventions aimed to reduce risk factors are needed. The effects on these factors were evaluated after interventions in either nutrition or physical activity in high school adolescents from Guadalajara, Jal., Mexico

Materials and Methods: 682 adolescents were assigned to either group with physical activity, diet or control. Variables studied before and after one year intervention were: insulin, glucose, insulin resistance (HOMA-IR), HDL-C, LDL-C, systolic and diastolic blood pressure, BMI, Tanner sexual development, physical activity, diet and tobacco use. Interventions were based on the social cognitive and constuctivist theories aimed to obtain significant learning and consisted of a course in either nutrition or physical activity, monthly counseling, workshops with parents and posters aimed to promote changes in behaviour. Homogeneity between groups was evaluated with ANOVA or X^2 and the effect of interventions with multiple linear regressions and a model of differences in differences.

Results: 248 adolescents comprised the control group, 227 the physical activity group, and 207 the diet group. Follow-up losses were 21.6%. Mean age at the start of the study was 15.8 years (range 14–19 years), 51.5% were female, 71.2% in low socieconomic status and 26.4% overweight. Initial insulin was 11.9±5.9 μU/ml, glucose: 85.6±7.2 mg/dl, LDL-C: 103.2±29.1 mg/dl, HDL-C: 48.0±12.0 mg/dl, systolic blood pressure: 114.3±10.6 mg/dl and diastolic blood pressure: 75.6±9.5 mg/dl. Physical activity reduced insulin concentration ($\beta=-1.62$, $p<0.01$), glucose ($\beta=-8.01$, $p<0.01$), HOMA-IR ($\beta=-0.59$, $p<0.01$), BMI ($\beta=-0.25$, $p<0.01$), systolic blood pressure ($\beta=-3.70$, $p<0.01$) and diastolic blood pressure ($\beta=-3.34$, $p<0.01$). Diet reduced BMI ($\beta=-0.39$, $p<0.01$) and diastolic blood pressure ($\beta=-3.83$, $p<0.01$).

Conclusion: Interventions in diet or physical activity are able to reduce risk factors for chronic diseases in high-school adolescents.

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0757

Identification of cardiovascular risk factors and long-term treatment of children and adolescents with overweight and obesity - a prospective trial with telemedical support (TeleObe)

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Background and Aims: During the last decades in children and adolescents the prevalence of overweight and obesity increased dramatically. It was the goal of the prospective trial to examine childrens' and adolescents' cardiovascular risk factors and to establish an effective strategy not only to reduce body weight, but also to induce a long-term stabilisation.

Materials and Methods: Out of a cohort of 228 overweight and obese children and adolescents (age 14,0± 2,4 ys, BMI 30,6±5,2 kg/m^2), addmitted to hospital to participate in a structured treatment and teaching programme (STTP) aiming weight reduction, 30 patients (13.2%) were screened positive with a cluster of metabolic and cardiovascular risk factors. All patients screened positive were included in a long-term treatment programme with telemedical support. It integrates data collection, visualization, and recommendations for handling by using mobile phone and Internet services. Its core is a module, which visualizes a summary of the patients' clinical data. A feedback from the physician to the patient is given periodically. At the end of the trial a questionnaire on patients experiences and satisfaction was applied.

Results: In children and adolescents (age 13.4±2.3 ys, BMI 32.1±6.0 kg/m^2) screened positive for cardiovascular risk factors the following parameters were identified: 11 patients (37%) had systolic blood pressure levels>120 mmHg, 5 (17%) had diastolic levels>90 mmHg (mean systolic/diastolic RR 119.5±12.9/75.2±9.9 mmHg), 12 (40%) had values of C-reactive proteine (CRP)>0.5 mg/dl (median 0.5 mg/dl), 22 (73%) had elevated uric acid levels (>381, median 449 μmol/l), 4 (13%) had a dyslipidemia and in 18 patients (60%) the carotis intima media thickness was above the cut-off (>0.47, median 0.50 mm). During the first phase of intervention, over 35.2±7.1 days patients participated in the STTP. BMI decreased to 29.9±4.7 kg/m^2 ($p<0,001$). Afterwards the telemedical support programme was started: During the follow-up of 12 months further modifications were registered in respect of eating habits in 73% of the patients, in physical exercise and sports in 60%. Parallel, there was still a tendency to further reduction of body weight ($p=0.079$). Using the telemedical support programme motivational and psychological problems were identified in 28% of the patients followed by feedback and specific interventions. All in all about 77% of participants considered telemedical support helpful for the everyday management and to improve empowerment and motivation.

Conclusion: In Western industry countries 10–20% of children and adolescents are overweight or obese. A high percentage has excessive risk factors for diabetes and cardiovascular diseases. Early intervention is mandadory. Hence, the telemedical support programme TeleObe seems to be a highly effective tool. It guarantees not only an effective initial weight reduction, but a stabilization over a long period

of time. Via feedback the identification and specific intervention is possible in respect of motivational, psychological or other problems in everyday management. TeleObe is highly accepted by children, adolescents and their parents.
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0758

Long-term aerobic exercise can potentiate the effect of rosiglitazone on islet preservation and insulin resistance in diabetic prone OLETF rat model

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Background and Aims: Steady aerobic exercise is recommended for diabetes treatment with anti-diabetic medications, particularly thiazolidinediones in obese type 2 diabetes.
Materials and Methods: We evaluated the effects of aerobic exercise (20 m/min, 30 minutes/day) and rosiglitazone (3 mg/kg/day) for 24 weeks on islet morphology and insulin resistance in obese diabetic prone animal model, OLETF rat.
Results: Increased lean body mass, decreased epididymal fat, and improved intraperitoneal glucose tolerance test were observed in both rosiglitazone plus exercise and exercise only groups (each group n=15). And the onset of diabetes was also delayed in both exercise and rosiglitazone group compared with control. On the chronological changes of islet morphology, more significant decrease of beta cell destruction was shown in rosiglitazone plus exercise group compared with exercise or rosiglitazone only. And exercise led to extension of normal expressions of insulin and GLUT-2 to the level of rosiglitazone group with western blot analysis and confocal microscopic finding, indicating comparable preventive or therapeutic effects of exercise with rosiglitazone treatment. The measured NOx (nitrite and nitrate) in pancreatic tissue were recovered in rosiglitazone only and plus exercise groups compared with control OLEFT rats.
Conclusion: We suggest the exercise is an essential regimen for islet preservation and improving insulin resistance in obese type 2 diabetes, particularly for anti-diabetic treatment with rosiglitazone.

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0759

Pharmacological and lifestyle interventions to prevent or delay type 2 diabetes mellitus in individuals with impaired glucose tolerance: a systematic review and meta-analysis

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Background and Aims: Individuals diagnosed with impaired glucose tolerance (IGT) have a high risk of developing type 2 diabetes (T2DM) and therefore many trials of interventions for prevention of T2DM have focused on such high risk individuals. Interventions assessed have been diverse but form two main categories, pharmacological or lifestyle. The aim of this study was to conduct a systematic review and meta-analysis of the effectiveness of pharmacological and lifestyle interventions, to prevent or delay T2DM, in individuals with IGT.
Materials and Methods: Medline and Embase databases were searched up to July 2005, for eligible randomised controlled trials (RCT). The reference lists of any studies that fitted our inclusion criteria and any relevant published reviews were checked. We selected RCTs published in English language that evaluated interventions to delay or prevent T2DM in individuals with IGT. Teams of reviewers independently extracted relevant data and assessed study quality using the Jadad score.
Results: Fifteen trials met the inclusion criteria for the systematic review: 13 trials comprising 7904 individuals with IGT, reported results in sufficient detail for inclusion in the meta-analyses. Due to the diversity of the interventions three separate meta-analyses were undertaken, all showed statistically significant benefit of intervention to delay or prevent T2DM. Results of the meta-analyses were, lifestyle interventions compared to standard advice, pooled hazard ratio 0.50 (95% confidence interval [CI]: 0.42, 0.59), oral hypoglycaemic agents compared to placebo, 0.66 (95% CI: 0.53, 0.81) and anti-obesity agent compared to placebo, 0.44 (95% CI: 0.28, 0.69).
Conclusion: Both lifestyle and pharmacological interventions significantly reduce the rate of progression to T2DM in individuals with IGT with lifestyle interventions proving just as effective as pharmacological.
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0760

Changes in insulin sensitivity in response to exercise in overweight Swedish and Arabian women with type 2 diabetes

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Background and Aims: Physical inactivity is directly linked to the rising incidence of insulin resistance and could contribute to the epidemic increase of type 2 diabetes in certain ethnic populations. Although exercise is frequently prescribed to patients with type 2 diabetes, there are few studies assessing the effect of exercise on insulin sensitivity in different populations. The purpose of this study was thus to determine the effects of exercise on insulin sensitivity and maximal aerobic capacity in previously sedentary, obese Swedish and Arabian women with type 2 diabetes.
Material and Methods: The study included 14 Swedish and 18 Arabian women (age 49.8±6.8) with type 2 diabetes (11 treated with insulin, 19 with oral agents and 2 with only diet) who participated in a supervised 6-month structured aerobic and resistance training program 2 times a week of moderate-intensity with the aim to increase insulin sensitivity by at least 20% and maximal oxygen uptake by approximately 10%. Insulin sensitivity (M-value) was measured by euglycemic hyperinsulinemic clamp combined with indirect calorimetry before and after 3 and 6 month intervention. Vo2max was estimated from submaximal ergometer test.
Results: All patients were markedly insulin resistant at entry to the study (M value approx. 30% of controls). Eleven Swedish and 9 Arabian completed the intervention. Vo2max at baseline was (19.9±5.1 ml/kgxmin) and increased by 13% after 3 months (22.7±4.8 ml/kgxmin, p=0.001) and 9% after 6 months (21.8±5.3 ml/kgxmin, p=0.018). M-value at the baseline was (2.75±1.6 mg/kgxmin) and increased by 10% after 3 month (3.03±2 mg/kgxmin, p=0.1) and 25% after 6 month (3.47±2 mg/kgxmin, p=0.007). This effect was primarily due to the Scandinavian group (p=0.001). BMI (32.6±3.8 vs. 32.5±4.1), HbA1c (6.49±1.3 vs. 6.39±1%), triglycerides (1.9±1.2 vs. 1.7±0.7 mmol/l), diastolic blood pressure (75.3±12 vs. 73.6±8.9 mmHg) were not significantly improved by training.
Conclusion: Exercise without weight loss increased modestly insulin sensitivity and maximal oxygen uptake in previously sedentary women with type 2 diabetes. However, the 25% enhancement in insulin sensitivity was not associated with a significant improvement in metabolic control suggesting that a longer or more intense training period and/or additional means are needed as treatment for type 2 diabetes.

PS 58

Mechanisms of obesity and weight loss

0761

Candidate gene polymorphisms for obesity and DM2: association with the somatotypes

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Background and Aims: Type 2 diabetes mellitus and obesity are complex diseases characterized by the interaction of the genetic and environmental factors. The aim of the study was to identify the possible associations of the selected candidate gene polymorphisms with somatotypes.
Materials and Methods: We genotyped polymorphisms in 7 different candidate genes for obesity or type 2 diabetes - INS VNTR (HphI), FABP2 (Ala54Thr), PPARgamma2 (Pro12Ala), B2AR (Gln27Glu), B3AR (Trp64Arg), UCP1 (A-3826G), Kir6.2 (Glu23Lys) in 301 unrelated well biochemically characterized non-diabetic subjects who underwent the oGTT and ITT tests. Detailed anthropometric data were taken using standard anthropometric technique according to Martin and Saller: 11 height measures, 14 circumferences, 12 width measures and 14 skin folds. Body composition (%fat mass, %muscles, %bone mass) was calculated using the ANTROPO programme. The anthropometric parameters were statistically analyzed after adjustment for age, men and women separately (NCSS 2004). To reduce the number of anthropometric variables, factor analysis was performed. This analysis revealed three main somatotype determining factors which were used in the following cluster analysis (K-Means).
Results: Three clusters of probands corresponding well to ectomorph, endomorph, and mesomorph somatotypes were obtained (number of subjects; BMI (kg/m^2); WHR; muscles/fat mass; presented as a mean±SD):
Ectomorphs women: (94; 23,26±4,21; 0,728±0,067; 1,74± 0,76); Ectomorphs men: (46; 26,40±3,40; 0,781±0,064; 2,49±0,98); Mesomorphs women: (25; 21,64±2,51; 0,721± 0,034; 3,04±0,43); Mesomorphs men: (31; 22,56±2,41; 0,767±0,028; 5,27±1,55); Endomorphs women: (65; 29,

84±4,54; 0,839±0,051; 1,37±0,61); Endomorphs men: (40; 32,94±5,37; 0,892±0,060; 2,51±0,71) With respect to cluster distribution, both genders were analyzed together according to genotypic frequencies. Among the assessed gene polymorphisms, UCP1 (A-3826G) displayed significant association with the defined somatotypes. The genotype frequencies: Ectomorphs AA(53,15%), AG(40,54%), GG(6,31%); Endomorphs AA(44,83%), AG(45,98%), GG(9,20%); Mesomorphs AA(60,9%), AG(29,0%), GG(10,1%) - Chi-square test, p=0,046.

Conclusion: We have demonstrated that the AA genotype of the UCP1 promoter polymorphism A-3826G is more frequent in the groups of lean probands (ectomorphs, mesomorphs) in comparison with probands who have abundant body fat content (endomorphs). It is in agreement with literature data reporting the G allele as obesity risk factor.

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0762

Neuromedin beta: the polymorphism P73T in Czech diabetic and nondiabetic populations

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Background and Aims: Neuromedin beta (*NMB*) is a member of the bombesin-like peptides widely expressed in brain, pancreas, adrenals and gastrointestinal tract. It regulates exocrine and endocrine secretions, smooth muscle contraction, feeding, blood pressure, glycemia, body temperature and cell growth. Recently, the significant association of the missense polymorphism P73T (c.217C>A) with the score of disinhibition and susceptibility to hunger was published. Homozygous mutation TT was associated with increased body weight, BMI, waist girth and body fat. The aim of our study was to determine the genotypic distribution of the *NMB* gene in the Czech diabetic and nondiabetic populations and to assess possible association of the minor allele T carriership with biochemical and anthropometric parameters.

Materials and Methods: Detailed biochemical and anthropometric characteristics of 288 patients with diabetes mellitus type 2 (DM2) (M/F 108/180; age 60±8.2 years; BMI 31.1±5,66 kg/m^2) and 367 nondiabetic subjects (ND) (M/F 132/235; age 32±11.0 years; BMI 23.7±3.82 kg/m^2) were collected. In healthy population the oGTT and insulin tolerance tests (ITT) were performed. For screening of P73T (c.217C>A) variant in the exon 2 of the *NMB* gene the single strand conformation polymorphism (PCR-SSCP) method was used. The positive controls (PP, PT and TT genotypes) were used in every run. Statistical analysis: Chi-square test, Mann-Whitney test and ANCOVA (adjustment for age) (NCSS 2004).

Results: The genotypic distribution (PP/PT/TT) in diabetic and nondiabetic populations was not significantly different: DM2 − 54.2/37.1/8.7 and ND − 51.0/41.1/7.9 [%]. In ND group the homozygotes for allele T had significantly higher level of glycosylated proteins (p=0.0007) that could be considered as an indicator of the eating behaviour. It is in agreement with previously observed relation of T allele to score of disinhibition and susceptibility to hunger. In DM2 patients the level of glycosylated proteins could be affected by diet and drug treatment. Nevertheless, we did not evidence the higher body weight, BMI, waist girth, body fat or any difference in other anthropometric parameters in T allele carriers. We noticed that T allele is associated with higher insulinemia. In DM2 the ratio fasting glycemia/fasting insulin (G_0/IRI_0) was significantly lower (p=0.0456) in T allele carriers. In ND the sum of fasting and stimulated insulin levels (ÓIRI0,60,120) and meanIRI0,60,120 were significantly higher (p=0.0424) in TT homozygotes and the area under the insulin curve in ITT (AUCITT) was significantly higher (p=0.0292) in T allele carriers.

Conclusion: Our study suggests that the polymorphism P73T of the *NMB* gene is associated with higher levels of glycosylated proteins and with higher insulin levels. *NMB* seems to be the candidate gene for the eating behaviour. However, we did not uphold its influence on the predisposition to obesity.

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0763

Changes of appetite regulation systems activity under a chronic social stress and experimental metabolic syndrome

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Background and Aims: Changes of appetite caused by action of unfavorable factors (including stress and unbalanced diet) evokes the increasing of the fat tissue mass of the body, which can lead to obesity, insulin resistance (IR) and metabolic syndrome (MS). The hormones of fat tissue - adipocytokines and endocannabinoid system plays the key role in appetite regulation. The aim of the present study was

to characterize the changes of appetite regulation systems activity under a chronic social stress and experimental MS in Golden Syrian hamsters.

Materials and Methods: 164 1-y-old Golden Syrian hamsters (males/females: 1/1) were randomized into 3 groups. A control group (n=12) was submitted to a standard diet. The 2nd group (n=76) was submitted for 5 weeks to a diet containing 29% vegetable- and animal oils and fructose (2 g/100 g b. w./day) - MS group. The 3rd group (n=76) was subjected for 5 weeks to the chronic social stress. For the final 3 days, 1, 2, 3, 4 and 5 weeks plasma leptin and adiponectin levels (by ELISA), anandamide (endogenic cannabinoid receptors ligand) levels in hypothalamus (by HPLC) were determined. Fasting blood glucose and insulin (by RIA) were measured. Homeostasis Model Assessment (HOMA) was used to estimate IR. Statistical analyses were carried out using SPSS and Microsoft Excel. Values are expressed as means±SEM.

Results: It has been shown, that both MS and stress are accompanied by body mass rising, but under stress the weight enhances after its fall. In both models were shown 2-phase changes of the leptin content: decrease during the first week and increase in 3 weeks. Inability of high dose of leptin to block the body mass increase indicated the leptin-resistance development. It was detected the strong correlation between leptin concentration and body mass rising in group 3 (males: r=0.70, p=0.045; females: r=0.86, p=0.018). IR was observed both in the models of MS and chronic stress. The plasma adiponectin level was decreased in the all time points of consideration. It was detected the strong correlation between adiponectin level and IR (group 2 males: r=−0.86, p=0.031; females: r=−0.73, p=0.044; group 3 males: r=−0.79, p=0.042; females: r=−0.92, p=0.026) also between body mass rising and IR under all experimental impacts. The orexigenic factor anandamide was significantly increase in hypothalamus of hamsters both under chronic social stress and experimental MS.

Conclusion: The hyperphagia against the background of body mass increase can be linked to leptin-resistance development and arrest of activation by leptin of orexigenic anandamide degradation. Our results suggest that changes of appetite which lead to obesity and insulin resistance development under stress and experimental metabolic syndrome have similar mechanisms.

0764

Assessment of abdominal subcutaneous and visceral adipose tissue volumes with multi detector-row computed tomography

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Background and Aims: Abdominal obesity is associated with insulin resistance, type 2 diabetes mellitus (T2DM) and cardiovascular diseases. Cross sectional imaging may enable accurate localization and quantification of subcutaneous and visceral adipose tissue. The reproducibility of multidetector-row computed tomography (MDCT) based volumetric quantification of abdominal adipose tissue and the ability to depict age and gender related characteristics of adipose tissue deposition have not been reported.

Materials and Methods: We evaluated a random subset of 100 subjects (age range: 37–83 years; 49% female) of the Framingham Heart Study offspring cohort who underwent MDCT scanning. Two readers measured subcutaneous and visceral adipose tissue volumes (SAV and VAV; cm^3) and areas (VAA and SAA; cm^2) as well as abdominal sagital diameter (SD) and waist circumference (WC).

Results: Inter-reader reproducibility was excellent (relative difference: −0.34%±0.52% for SAV and 0.59%±0.93% for VAV, intra class correlation (ICC)=0.99 each). The mean SAA/VAA ratio was significantly different from the mean SAV/VAV ratio (2.0±1.2 vs. 1.7±0.9; p<0.001). The ratio of SAV/VAV was only weakly inversely associated with SD (ICC=−0.32, p=0.01) and not significantly associated with WC (ICC=−0.14, p=0.14) or BMI (ICC=−0.17, p=0.09). The mean SAV/VAV ratio was significantly different between participants <60 years vs.>60 years (1.9±1.0 vs. 1.5±0.7; p<0.001) and between men and women (1.2±0.5 vs. 2.2±0.9; p<0.001).

Conclusion: This study demonstrates that MDCT-based volumetric quantification of abdominal adipose tissue is highly reproducible. In addition, our results suggest that volumetric measurements can depict age and gender related differences of visceral and subcutaneous abdominal adipose tissue deposition. Further research is warranted to assess whether volumetric measurements may substantially improve the predictive value of obesity measures for insulin resistance, T2DM and other diseases.

0765

Osteoprotegerin declines in parallel with insulin resistance and inflammatory biomarkers in morbidly obese patients after weight loss

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Background and Aims: Morbid Obesity is associated with chronic inflammation, insulin resistance and an increased risk for development of diabetes and cardiovascular mortality. Recent studies suggest that osteoprotegerin (OPG) may play an important pathogenetic role in the atherothrombotic complications in cardiovascular disease as well as in diabetes. Thus, it was of interest to study OPG in insulin resistant patients with morbid obesity before and after massive weight loss induced by bariatric surgery.
Materials and Methods: A total of 32 patients (mean age 41±12 years) with morbid obesity was studied before and 26.3±11.4 months after bariatric surgery. High sensitivity assays were used to measure concentrations of fasting OPG, monocyte-chemoattractant-protein-1 (MCP-1), Interleukin-18 (IL-18), and high-sensitive C-reactive protein (hsCRP).
Results: After a mean weight loss of 34.1±17.0 kg, circulating OPG decreased significantly from 4.7±1.6 to 2.7±1.4 ng/ml ($p<0.001$), hsCRP from 0.8 (0.3–1.4) to 0.1 (0.1–0.2) mg/dl ($p<0.001$), MCP-1 from 576±185 to 314±149 pg/ml ($p<0.001$) and IL-18 from 1478±631 to 981±591 pg/ml ($p=0.001$). The decline in OPG after weight loss correlated significantly with the decrease in body-mass-index, fasting insulin, HOMA-IR, and the inflammatory biomarkers MCP-1 and hsCRP.
Conclusion: This is the first intervention study demonstrating a significant decrease in circulating OPG in association with an improvement of both insulin resistance and chronic inflammation in morbidly obese patients after weight loss. Since high OPG was shown to predict cardiovascular death and myocardial infarction in several prospective studies, the observed marked lowering of OPG might be of clinical relevance in morbidly obese insulin-resistant patients.

0766

Visceral but not subcutaneous fat reduction is responsible for metabolic improvements (MI) after weight loss (WL)

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Background and Aims: Visceral adiposity associates with insulin resistance (IR) and increased cardiovascular risk (CVR). Inflammatory cytokines levels are increased in obese subjects and might be responsible for IR and increased CVR. WL is associated with improved insulin sensitivity (IS) and decreased inflammatory cytokines levels, however, little data exist about the relative role of visceral and subcutaneous fat loss on the MI brought about by WL. Aim of this study was to investigate whether, after caloric restriction, changes in IS and in TNF and IL6 plasma levels correlate with visceral and/or subcutaneous fat loss.
Materials and Methods: We determined in 10 obese subjects (BMI 33.8 2.5 Kg/mq) visceral and subcutaneous abdominal fat volume (VAV and SCV) by MRI. Subjects were enrolled in a WL program based on dietary and life style counselling and MRI was repeated after each subject had achieved a WL equal to at least 5% of the original BW. Fasting plasma glucose, insulin, TNF, IL6 and leptin were measured before and after WL.
Results: After WL, IS significantly improved ($p<0.05$) while TNF, IL6 and leptin levels decreased (by 40%, 45% and 30% respectively, $p<0.05$ for all). We observed a significant correlation ($r=0.64$, $p<0.01$) between the amount of VA lost and the improvement in IS, between the percent reduction in VAV and the percent reduction in both TNF ($r=0.71$, $p<0.01$) and IL6 ($r=0.61$, $p<0.01$) plasma levels. No correlation was observed between the investigated parameters and either the amount or the percentage of SCV lost.
Conclusion:The reduction in VAV but not in SCV may be responsible for MI after WL in obese subjects.

0767

Increase of visfatin after weight loss induced by gastroplastic surgery

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Background and Aims: The recently described adipocytokine visfatin is produced in visceral fat and has been suggested to influence insulin resistance. For a further investigation of the relationship between visfatin and insulin resistance this adipocytokine was measured in insulin resistant morbidly obese patients before and after gastroplastic surgery.
Materials and Methods: Visfatin, interleukin (IL)-6, high sensitivity C-reactive protein (hsCRP), homeostasis model assessment insulin resistance (HOMA-IR) and other clinical

parameters were assessed in 36 morbidly obese subjects (28 female, mean age 43 yrs.) with a median body mass index (BMI) of 44.3 kg/m2.
Results: Median (95% confidence interval) visfatin concentrations increased significantly after weight loss (70.9 [61.4–75.6] ng/ml vs. 86.4 [79.4–89.8] ng/ml; p<0.0005). This increase correlated with the decrease of insulin and HOMA-IR and was paralleled by a reduction of IL-6 and hsCRP plasma concentrations after gastroplastic surgery.
Conclusion: Massive weight loss after gastroplastic surgery is accompanied by an increase of the novel adipocytokine visfatin. This increase correlates with the decrease of insulin plasma concentrations and HOMA-IR.

0768

Comparision of the effects of peroxisome proliferator-activated receptor-γ with -α on energy homeostasis and fat distribution in high-starch-fed rats

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Background and Aims: Peroxisome proliferator-activated receptor (PPAR)-α activation enhances free fatty acid oxidation and PPAR-γ activation is involved in adipocyte differentiation, fatty acid uptake and storage. This study focused on the effects of PPAR-α and -γ activation on energy homeostasis and fat distribution in high-starch-fed rats.
Materials and Methods: Weight gain, food intake, feeding efficiency, and metabolic surrogate markers were analyzed in high-starch-fed rats treated for 8–10 weeks with either PPAR-α activator fenofibrate or PPAR-γ activator pioglitazone, and compared with high-fat-fed rats. Body fat distribution was evaluated in subcutaneous and visceral fat using a computed tomography scan at upper margin of pelvis level.
Results: High-starch feeding significantly raised the level of TG and high-fat feeding raised the level of TC. Pioglitazone increased and fenofibrate decreased caloric intake. Body weight gain increased in pioglitazone-treated rats and decreased in fenofibrate-treated rats. PPAR-γ activator markedly increased feeding efficiency, whereas PPAR-α agonist treatment decreased feeding efficiency. Furthermore, PPAR-γ activator tended to increase subcutaneous fat area/visceral fat area (SFA/VFA) ratio.
Conclusion: PPAR-γ activation is associated with weight gain due to both increased food intake and improved feeding efficiency, whereas PPAR-α activation leads to opposite effects. The actions were independent of dietary content. There was a redistribution of fat from intra-abdominal visceral region to abdominal subcutaneous region by pioglitazone.

0769

Weight reduction decreases TNF-α and increases IL-6 gene expression in lymphocytes in subjects with metabolic syndrome - the GENOBIN study

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Background and Aims: Obesity has been considered as a proinflammatory state. Inflammation has been implicated in metabolic syndrome (MS), type 2 diabetes and cardiovascular diseases. Weight loss may have an effect on expression of genes related to inflammation in adipose tissue, and genes like tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-6 have been found to be increased in the adipose tissue of obese subjects. However, expression of these genes in immune cells is poorly described. We sought to evaluate how long-term weight reduction affects expression of genes related to inflammation in lymphocytes.
Materials and Methods: We carried out a randomized clinical trial in 75 40–70 year-old overweight or obese men and women with the MS. The subjects entered one of three lifestyle interventions - weight reduction (WR), aerobic training or resistance training - or the control group. Subjects randomized to WR participated in an intensive 12 wk weight loss period in which they met a clinical nutritionist for 5 times. During the following weight maintenance phase 13–34 wks, they met the clinical nutritionist twice. Blood samples were taken at the beginning, at wk 12 and at the wk 34. Lymphocytes from 24 subjects from the WR group intervention were isolated with the use of Lymphoprep kit. Total RNA was extracted and converted to cDNA for quantitative real-time PCR gene expression analysis. The Ethics Committee of Kuopio University Hospital approved the protocol according with the standards of the Helsinki Declaration.
Results: A significant reduction in weight (P<0.001), waist circumference (P<0.001), body fat (BF; P<0.001), BF% (P<0.001) and fasting plasma glucose (FPG; P<0.001) was observed in the WR group at wk 34 compared with wk 0. Gene expression levels of TNF-α were lower at wk 34 than at wk 0 [median 23.4% (95% CI: 1–24), P<0.05]. Conversely, IL-6 expression increased at wk 34 compared with wk 0 [median 9% (95% CI: −5–35), P<0.05]. Changes in both genes were significantly correlated between each other (r=0.61, P=0.002). Change in IL-6 expression was significantly correlated to changes in BF

(r=−0.42, P=0.042), in BF% (r=−0.49, P=0.014) and in FPG (r=−0.44, P=0.035). Even when adjusting for changes in weight or waist circumference, correlations between changes in IL-6 expression with FPG and BF changes remained significant.

Conclusion: Weight loss in subjects with MS decreased TNF-α gene expression and paradoxically increased IL-6 gene expression in lymphocytes. The increase of IL-6 expression was correlated with decreases in body fat and fasting plasma glucose levels. Although the literature seems to support a decrease in gene expression of proinflammatory cytokines such as IL-6 with weight reduction, the immunomodulatory effects of weight loss on gene expression in lymphocytes may be more complex.

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Metabolic effects of exercise

0770

Effect of short-term exercise training on insulin action and muscle energy metabolism

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Background: Exercise training increases whole body insulin sensitivity due to increased muscle glucose transport in healthy and insulin resistant humans. In insulin resistant humans, such as elderly or 1st degree relatives of type 2 diabetic (T2D) patients, reduced flux through ATP synthase mainly contributes to impaired muscle glucose transport and to elevated hepatocellular fat content (HCL). Thus, we hypothesized that exercise training should increase ATP synthetic flux in parallel with improvement of glucose metabolism.

Materials and Methods: Healthy humans (n=7; age: 40±5 a, body mass index, BMI: 22±1 kg/m^2) without a family history of T2D underwent a 75-g oral glucose tolerance test (OGTT) and *in vivo* magnetic resonance spectroscopy (MRS). Thereafter, they performed incremental exercise testing (+10–20 W/min until exhaustion on a cycle ergometer, respiratory gas exchange measured in breath-by-breath mode using an open-air spirometry system) and further two bouts of exercise (40 min, 90% of power output at the respiratory compensation point, rcp, on a cycle ergometer) within one week. At 48 h after the last bout of exercise, OGTT and MRS were repeated. Areas under the time curves (AUC) of glucose, insulin and C-peptide (cp) concentrations and model analyses were performed to derive parameters of insulin action and secretion (OGIS, adaptation index). Rates of muscle ATP synthesis were measured with the saturation magnetisation transfer experiment using ^{31}P MRS (n=6). HCL were quantified with ^{1}H MRS. Lactate concentrations in arterialized blood were measured with an enzymatic-amperometric method to allow for determination of rcp (Wasserman, 1984).

Results: Incremental exercise testing revealed VO2max and VO2rcp of 30.8±3.1 and 23.0±2.2 ml.kg^{-1}.min^{-1}. Fasting glucose (88±3 vs. 87±3 mg/dl) and insulin concentrations (3.6±0.5 vs. 3.4±0.3 mU/l) were comparable before and after exercise training. Short-term exercise training augmented the initial C-peptide release (AUCcp30, before vs. after: 119±9 vs. 132±6 ng/ml.30 min; p<0.01). The adaptation index markedly increased ({OGIS x dAUCcp30/dAUCg30}, before vs after: 183±33 vs. 277±69; p<0.05). Insulin sensitivity only tended to increase (OGIS, before vs after: 451±9 vs. 475±17 ml.min^{-1}.m^{-2}; p<0.076). Exercise training increased muscle ATP synthesis by 22% from 11.5±0.9 to 14.0±0.9 μmol.g^{-1}.min^{-1} (p<0.005), whereas HCL remained unchanged (before vs. after: 1.44±0.38% vs.1.29±0.29%, ns)

Conclusion: Short-term exercise training at near maximal exercise capacity increases β-cell function and adaptation of β cell function to insulin sensitivity. Flux through muscle ATP synthase rises before marked changes in insulin sensitivity suggesting that exercise training primarily improves mitochondrial function.

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0771

Substrate source utilization in master athletes compared to healthy sedentary controls

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Background and Aims: Endurance exercise training has been associated with increased skeletal muscle fat oxidative capacity. In accordance, greater whole-body fat oxidation rates have been reported at rest and/or during exercise conditions in the endurance trained state. However, data on the specific substrate sources that are being utilized to a greater or lesser extent in the endurance trained state (i.e. plasma derived glucose, muscle glycogen, plasma free fatty acids (FFA), or intramuscular triacylglycerol (IMTG)) are inconclusive.

Materials and Methods: In the present study, we applied contemporary stable isotope methodology to determine whole-body substrate source utilization rates at rest, during moderate intensity exercise (50% Wmax) and recovery in 10 master athletes and 10 healthy, age-matched sedentary controls (age: 57±1 and 60±2 y; BMI: 26±1 and 28±1; VO_2-max: 48.8±1.8 and 36.8±2.0 $ml \cdot kg^{-1}$ body weight, respectively). [U-^{13}C]palmitate and [6,6-$^2H_2$]glucose tracers were applied to assess plasma FFA and glucose oxidation rates, and to estimate the use of muscle and/or lipoprotein derived triacylglycerol and muscle glycogen. Muscle biopsies were taken before and after exercise to assess net changes in muscle fiber type specific lipid and glycogen content.

Results: At rest, plasma palmitate rate of appearance (Ra), rate of disappearance (Rd) and rate of oxidation (Rox) were not significantly different between groups. During moderate-intensity exercise (150±5 (T) and 101±8 W (C)), plasma palmitate Ra, Rd and Rox, were greater in trained subjects compared to the control group ($P<0.001$). The latter was accompanied by a significantly greater total fat oxidation rate (0.52±0.03 $g.min^{-1}$ and 0.35±0.03 $g.min^{-1}$ in T and C, resp., $P<0.001$), contributing 43±2 and 39±3% to total energy expenditure during exercise ($P=0.19$, NS). Elevated fat oxidation rates were mainly attributed to a greater plasma FFA oxidation rate, both from an absolute ($P<0.002$) as well as a relative point of view ($P=0.06$). Muscle plus lipoprotein derived TG use contributed only 10±2 and 11±3% in the trained and control group during exercise (NS). No significant changes in net type I or II muscle fiber lipid content were observed following exercise in both groups. Concomitantly, total carbohydrate oxidation rates during exercise were reduced in trained subjects as compared to sedentary controls (1.70±0.07 $g.min^{-1}$ and 1.37±0.08 $g.min^{-1}$ in T and C, resp., $P<0.01$). The reduced carbohydrate oxidation rates were mainly attributed to a decline in plasma glucose utilization rate, averaging 8±0 and 14±1% in trained vs. control subjects ($P<0.001$), whereas the relative contribution of muscle glycogen did not differ between groups (49±2 and 46±3% in T and C, resp.).

Conclusion: In conclusion, master athletes oxidize significantly more plasma FFA and less plasma glucose as compared to sedentary, age-matched controls, at the same relative exercise intensity. IMTG stores do not seem to represent a major substrate source during moderate intensity exercise in either sedentary or endurance trained middle-aged males.

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0772

Response of insulin-treated long-term type 2 diabetic subjects to a 5-month exercise training programme

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Background and Aims: Insulin resistance is the key player in the pathogenesis and progression of type 2 diabetes (T2D), leading to a severely impaired blood glucose homeostasis. Recently, mitochondrial dysfunction has been hypothesised to play a role in the development of skeletal muscle insulin resistance, leading to a reduced ability to oxidize fatty acids and to an accumulation of intramyocellular lipids (IMCL). Exercise training, in various forms, can influence IMCL, mitochondrial function, as well as insulin sensitivity and has the potential to be an efficient intervention in the prevention as well as the treatment of T2D.

We studied this triad of IMCL, skeletal muscle mitochondrial function and glucose homeostasis in insulin-dependent T2D patients before and after 5 months of combined strength and endurance exercise training.

Materials & Methods: Eleven male T2D patients were selected (mean±SE: age: 59.1±2.3 years, BMI: 32.2±1.2 kg/m^2). Subjects were diagnosed with T2D over 5 years and were on exogenous insulin treatment for at least 24 months. Subjects participated in a supervised 5-month progressive circuit-training program consisting of 45–60 min of resistance and interval exercises for 3 times a week. Before and after the 5-month training program the following parameters were measured: fasting plasma glucose and HbA_1c concentration, maximal whole-body oxygen capacity (VO_{2max}) and maximal workload (P_{max}) on a cycle ergometer, body composition (DEXA), IMCL using ^{1}H MR spectroscopy (MRS) and post-exercise phosphocreatine (PCr) and ADP recovery time constants (τ_{PCr} and τ_{ADP}) applying dynamic ^{31}P MRS in the *m. vastus lateralis*.

Table 1: Body composition, blood glucose homeostasis, IMCL content and ^{31}P parameters before and after 5 months of exercise training

	BM	WC	HbA_1c	Fat	FFLM	Fasting plasma glucose	VO_{2max}	P_{max}	IMCL	τp_{Cr}	τ_{ADP}
	kg	cm	%	%	kg	mmol/l	ml/min/kg	Watt/kg BM	% of water signal	s	s
PRE training	97.5±4.9	112.6±3.7	7.6±0.3	27.0±0.8	20.6±1.0	10.4±0.9	24.3±1.4	1.6±0.1	2.05±0.28	49.44±5.48	22.47±2.86
POST training	97.5±4.8	113.2±4.0	7.2±0.2	25.9±0.9	21.2±0.9	8.6±0.7	24.2±1.5	1.9±0.2	2.40±0.43	45.56±5.60	21.19±2.45
p-value	0.95	0.69	0.04	0.009	0.033	0.059	0.95	0.0004	0.107	0.088	0.434

BM: body mass, WC: waist circumference, HbA_1c: glycolysated haemoglobin, Fat: whole body fat percentage, FFLM: fat free leg mass, VO_{2max}: maximal oxygen consumption, P_{max}: maximal power output, IMCL: intramyocellular lipid, τ: metabolite's time constant of recovery

Results: Table 1 shows the parameters measured before and after the exercise-training program. Body mass (BM) or waist circumference (WC) of the subjects remained unchanged after 5 months of exercise training. However, whole body fat percentage and fat free leg mass (FFLM) both changed significantly. The plasma marker of glucose homeostasis, HbA_1c, was significantly improved and fasting plasma glucose levels tended to be lower after the exercise-training period. VO_{2max} did not change due to the exercise training, whereas P_{max} increased. ^{1}H MRS measurements revealed no differences between IMCL levels before and after the training program. Also ^{31}P parameters of oxidative capacity, τ_{PCr} and τ_{ADP}, determined from the dynamic ^{31}P MRS measurements showed no improvement. **Conclusion:** In these T2D patients suffering from severe muscle deconditoning, the exercise training did result in an improved glucose homeostasis, indicated by the lower HbA_1c and a trend towards lower fasting plasma glucose. This was not accompanied by an amelioration of mitochondrial function (VO_{2max}, τ_{PCr} and τ_{ADP} did not change) or a decrease in IMCL content, although maximal workload per kg BM did increase.

0773

Leptin and adiponectin responses following acute resistance training in aged males

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Background and Aims: Adiponectin and leptin are closely related to weight control and insulin sensitivity. Exercise affects elderly metabolic regulation and functional capacity. The purpose of this study was to investigate leptin and adiponectin responses in elderly males following acute resistance exercise training.
Materials and Methods: Forty inactive men (65–82 years) were randomly assigned to a control (C, n=10), low-intensity training (LI/n=10), moderate-intensity training (MI/n=10), and high-intensity training (HI/n=10) group. Strength, exercise energy cost (EEC), VO_2max, resting metabolic rate (RMR), plasma leptin and adiponectin, were determined at baseline, immediately following exercise, and at 12−, 24−, 48−, and 72-hours post-exercise. Statistical analysis was performed using repeated measures ANOVA.
Results: Following exercise leptin decreased in all active groups, effect being significant only in the HI group (P<0.05). Furthermore both % (HI: −10.62±1.99, MI: −6.68±6, LI: −4.94±3.75, C: 11.4±5.7, p=0.014) and absolute decrease of leptin in all active groups was significantly different compared with controls. Adiponectin levels increased significantly only in HI group (p<0.001), effect preserved even 12 h following exercise. Furthermore both % (HI: 71.2±13.8, MI: 13±9, LI: 9.6±11.6, C: −0.89±11.4, p=0.003) and absolute increase of adiponectin in the HI

group was significantly higher compared with all other groups.
Conclusions: Acute resistance exercise training may alter leptin and adiponectin levels in an intensity-dependent manner.

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Hyperinsulinaemia during exercise does not suppress hepatic glycogen utilisation in patients with type 1 diabetes

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Background and Aims: Patients with type 1 diabetes run the risk of hypoglycaemia during exercise. It has been suggested that this may be due to impaired mobilisation of liver glycogen associated with relative hyperinsulinaemia. The aim of this study was to compare the dynamic in-vivo changes in liver glycogen and whole body substrate metabolism in patients with type 1 diabetes and healthy volunteers during exercise using ^{13}C magnetic resonance spectroscopy and indirect calorimetry.
Methods: 7 male patients with uncomplicated type 1 diabetes [Group D, Mean (SEM) diabetes duration 10 (2) yr, age 33 (3) yr, BMI 24 (1) kg/m^2, HbA_{1C} 8.2 (0.2)% and VO_{2max} 49 (2) ml.kg^{-1}.min^{-1}] and 5 non diabetic controls [Group C, age 30 (3) yr, BMI 22 (1) kg/m^2, HbA_{1c} 5.4 (0.1)% and VO_{2max} 58 (4) ml.kg^{-1}.min^{-1}] were studied before and after a standardised breakfast (2.5 g carbohydrate/kg) and after 3 bouts (EX1, EX2, EX3) of 40 min cycling at 60% VO_{2max}.The interval between breakfast and the first bout of exercise was ~212 h. Patients were admitted overnight, having omitted their long acting insulin, and euglycaemia was maintained with a variable low dose insulin infusion. Patients subcutaneously injected 0.5 units of rapid acting insulin analogue (Novorapid) per 10 g of carbohydrate with breakfast. Furthermore, insulin was infused throughout the trial at a rate of 5 mU/m^2/min for basal requirements. ^{13}C glycogen measurements were acquired in a 3.0T magnet using a surface coil. The C1-glycogen peak at 100.4 ppm was analysed. Liver glycogen concentrations were quantified using a phantom of known glycogen concentration. Whole body substrate oxidation was determined during a separate visit under identical conditions.
Results: Fasting glucose [6.1(0.9) vs. 3.7 (0.2) mmol/l] and insulin [26 (8) mU/l vs.7 (2)] concentrations were higher in D than C respectively ($P<0.05$). Glucose and insulin concentrations were higher ($P<0.01$) during EX1 in D [11 (2) mmol/l and 34 (7) mU/l respectively] compared with C [3.0 (0.1) mmol/l and 5 (1) mU/l respectively]. Insulin concentrations declined during the remaining two exercise sessions in D [EX2: 25 (3) mU/l and EX3: 22 (2)] but were still greater ($P<0.01$) than C. There were no differences in fasting liver glycogen between groups [D: 178 (58) mmol/l vs. C: 212 (84)]. Post-prandial glycogen repletion was also similar [D: 589 (169) vs. C: 463 (117)]. Liver glycogen concentrations after each exercise session in D were comparable to C [EX1: 298 (87) vs. 303 (64), EX2: 267 (142) vs. 210 (65) and EX3: 195 (98) vs. 145 (66) respectively] despite the relative hyperinsulinaemia. Whole body carbohydrate oxidation during exercise in D was similar to C [2.1(0.2) g/min vs 2.3 (0.2)].
Conclusion: Hyperinsulinaemic conditions during exercise in patients with type 1 diabetes do not suppress hepatic glycogen utilisation. This does not support the perception that exercise-induced-hypoglycaemia in patients with type 1 diabetes is secondary to suppressed hepatic glycogen utilisation.
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Metabolic effects of physical exercise started at an advanced age

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Background and Aims: The proportion of elderly increases in Westernized societies, which is associated with an increase in type 2 diabetes and cardiovascular disorders. Weight reduction and physical exercise are effective strategies for the prevention of these diseases, but only few data are available about the efficiency of life-style programs when started at an advanced age.
Materials and Methods: We have restrospectively evaluated clinical and laboratory data of 580 patients older than 50 years of age, who were admitted to our medical center for a bicycle exercise test. Beside the results of this test (percentage of maximal physical capability), patients were asked about the performance of regular physical exercise,

and whether they have started before or after the age of 50 years. Medication was recorded in all patients

Results: 301 patients (mean age 68.6+/−22.0 years) reported about regular physical exercise, 106 (mean age 67.8+/−7.0 years) had started after an age of 50 years. 279 patients (mean age 69.8+/−18.0 years) denied the performance of physical exercise. The corresponding maximal capability in the exercise test was 94+/−23% in the active and 80+/−22% in the inactive group (p<0.001). Body mass index (BMI) (25.3+/−3.1 kg/m2) and the percentage of body fat (30.4+/−6.5%) were significantly lower in the active than in the inactive group (BMI 27.7+/−3.7 kg/m2; body fat 35.1+/−6.3%;p<0.001). This was paralleled by a more favourable metabolic risk profile. C-reactive protein values were 0.24+/−0.09 mg/dL in inactive patients and 0.22+/−0.08 mg/dL in active (p<0.001). Between patients, who had started regular physical exercise before or after the age of 50 years, only the percentage of body fat revealed a significant difference (29.6+/−6.6% and 31.3+/−6.1%; p<0.010). The frequency of cardiovascular and metabolic medication was significantly lower in the active group, but did not differ between patients who started exercise before or after the age of 50 years. Among the 135 diabetic patients, 58 (mean age 75.0+/−7.8 years) reported to be physically active and 78 (mean age 74.6+/−8.0 years) to be inactive. Active diabetic patients revealed significantly lower HbA1c values (6.7+/−1.2%), systolic (144+/−21.5) and diastolic (83+/−11) blood pressure values than inactive patients (HbA1c 7.1+/−1.3%;p<0.001; systolic blood pressure 149+/−20; p<0.001: diastolic blood pressure 86+/−11; p<0.010).

Conclusion: Regular physical exercise, also when started beyond an age of 50 years, is related to a more favourable metabolic risk profile and lower rate of medication compared to inactive patients at the same age.

0776

Effects of exercise on inflammatory markers, insulin resistance, and arterial stiffness

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Backgrounds: Physical exercise exerts many beneficial effects. It protects against diseases associated with chronic low-grade inflammation, such as insulin resistance and atherosclerosis. But acute bouts of intense or prolonged strenuous exercise such as marathon running can stimulate inflammatory response. It may trigger acute myocardial infarction, sudden cardiac death, immunosuppression, oxidative stress, and biochemical and hormonal changes.

Aims: The objective of this study was to ascertain whether exercise in ultra-marathon runners affects inflammatory markers, insulin sensitivity, and arterial stiffness.

Methods: Study populations were ultra-marathon hobby runners who participated in 622-km ultra-marathon foot race held July 10 to July 16, 2005 in Korea. Twenty ultra-marathon runners consented to this study. Only 13 participants were studied pre-race, immediately after the race, and 4 weeks after the race. We also compared 20 ultra-marathon runners with age-, sex-, and BMI-matched 20 healthy control subjects. We measured lipid profiles, glucose, insulin, adiponectin, and inflammatory markers. Insulin resistance was calculated by Homeostasis Model Assessment (HOMA). Total body fat mass was measured using a multiple frequency bioelectrical impedance method. We also measured brachial-ankle pulse wave velocity (baPWV).

Results: Ultra-marathon foot race elevated plasma glucose (P=0.007), insulin (p=0.001), hsCRP (P=0.039), IL-6 (P=0.016), HOMA-IR (P=0.003), liver enzyme and muscle enzyme. It reduced total cholesterol (P<0.001), triglyceride (P<0.001), LDL-C (P<0.001) and body weight (P=0.003). But all these parameters were returned to the baseline values within 4 weeks after the race except insulin and HOMA-IR. Ba-PWV and body fat mass were not changed significantly. In a multiple regression analysis, the elevation of liver enzymes in the pre-race predicted the elevation of muscle enzymes after ultra-marathon race.

Compared with the healthy controls, there were no difference in adiponectin, TNF-α, hs-CRP, total cholesterol, triglyceride, and HDL-cholesterol. But there were difference in body fat mass, the amount of exercise, IL-6, insulin, HOMA-IR, LDL-cholesterol, and ba-PWV between the two groups.

Conclusions: The present study shows that regular exercise decrease body fat mass. Also it significantly increases IL-6. But TNF-α is insignificantly decreased. The changes of body fat mass, inflammatory markers, and lipid profiles by regular exercise affect insulin sensitivity and arterial stiffness. It also shows that extreme exercise influences biochemical parameters, hormonal levels, inflammatory markers, and insulin resistance. Insulin resistance particularly is related to the elevation of inflammatory cytokines and sub-clinical damages of muscle and liver. But the influences of ultra-marathon foot race are transient and reversible within 4 weeks after the race except insulin and HOMA-IR.

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0777

At high insulin levels, intravenous AICA-riboside infusion does not affect whole body nor forearm skeletal muscle glucose uptake *in vivo* in humans

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Background and Aims: AMPK is a potential pharmacological target for the treatment of type 2 diabetes. 5-Aminoimidazole-4-carboxamide-riboside (AICA-riboside) activates AMPK. Animal studies have shown that AICA-riboside stimulates glucose uptake in skeletal muscle and improves insulin sensitivity. In a previous human *in vivo* study, we showed that intra-arterial AICA-riboside is a potent vasodilator and decreases systemic glucose and free fatty acid levels, but it has no direct local effect on skeletal muscle glucose uptake. In the present human *in vivo* study we investigated whether AICA-riboside infusion increased whole body glucose uptake during hyperinsulinemia.
Materials and Methods: Studies were performed in healthy subjects (n=15, age: 21.6±2.2 yr, BMI 21.4±1.5 kg/m^2). They underwent two euglycemic hyperinsulinemic (60 mU/m^2/min) clamps with and without systemic co-infusion of AICA-riboside, in a single-blind, randomized order. After 60 min of the clamp, insulin was combined with AICA-riboside (66.7 mg/kg) or placebo (=normal saline) infusion for an additional 60 min. Forearm blood flow (plethysmography) and plasma AV glucose difference were used for calculation of Forearm Glucose Uptake (FGU). In addition, (intra-arterial) blood pressure and heart rate were measured. We measured AICA-riboside plasma concentrations and AICA-riboside uptake and phosphorylation to ZMP in erythrocytes using HPLC technique.
Results: During insulin with AICA-riboside or placebo infusion, insulin levels increased almost equally (474±25 and 471±17 pmol/l resp.). Glucose infusion rates (95–120 min) were also similar (48.5±7.7 and 45.5±4.3 μmol/kg/min resp., *P*=NS between both groups). Insulin with AICA-riboside had no effect on FGU versus insulin with placebo (0.2±0.1 to 2.2±0.6 and 0.4±0.1 to 2.3±0.4 μmol/min/dl resp., *P*=NS). AICA-riboside slightly increased FBF (from 1.2±0.2 to 1.6±0.3 ml/min per dl of forearm tissue) compared to placebo (1.4±0.2 to 1.2±0.2 ml/min/dl, $P<0.05$). AICA-riboside increased heart rate from 60±4 to 74±3 versus 62±4 to 66±4 bpm during placebo ($P<0.05$). Mean arterial blood pressure tended to decrease during AICA-riboside compared to placebo (83±2 to 80±2 and 83±2 to 84±2 mmHg resp., *P*=0.09). The AICA-riboside plasma concentration increased from 0±0 to 203.6±5.8 μM during AICA-riboside infusion (n=3). There was also a significant increase in AICA- riboside uptake and phosphorylation to ZMP in erythrocytes.
Conclusion: During hyperinsulinemia, AICA-riboside does not increase insulin-stimulated whole body nor local skeletal muscle glucose uptake in humans. AICA-riboside induces systemic vasodilation in skeletal muscle resulting in an increase in heart rate and decrease in blood pressure. These results are in line with our earlier findings, and suggest that in humans, AICA-riboside may not reach the subendothelial compartment (skeletal muscle) and thus may not be able to stimulate glucose uptake. AICA-riboside is probably taken up and phosphorylated in the endothelium, resulting in profound changes in vascular tone. This current study does not allow to determine whether AICA-riboside acts in the liver. Because here the endothelial junctions are less tight, AICA-riboside may be able to inhibit hepatic glucose production and hence lower plasma glucose levels.
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0778

Effect of body composition on the validity and reliability of an electronic pedometer

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Background: Pedometer based activity recommendations such as the 10,000 steps programme, are increasingly being used to promote physical activity and subsequently control weight in people with Type 2 diabetes. While pedometer validity has been confirmed in individuals with a normal body composition, there is conflicting evidence about pedometer validity in overweight and obese individuals.
Aims: 1) examine the effect of body composition on the validity and reliability of the Yamax Digiwalker SW 401 pedometer while walking at three self-selected walking speeds; 2) examine the effect of different pedometer placement sites on the validity and reliability of the pedometer.
Material and Methods: 52 adults (24F, 28M) participated with Body Mass Index (BMI) ranging from 21 to 52 kg/m^2. Body composition was measured by BMI, waist to hip ratio and % body fat (Harpenden skin fold callipers, 4 placement sites) and categorised as follows: BMI (category 1–20–24.9 kg/m^2 (n=11), category 2–25–29.9 kg/m^2 (n=15), category 3–30–34.9 kg/m^2 (n=13), category 4–35 kg/m^2 and over (n=13); waist/hip ratio (category 1<0.8 for women, 0.9 for men (n=17), category 2>0.8 for women, 0.9 for men (n=12), category 3>0.85 for women, 1 for men (n=23). Percent body fat was categorised using reference table adapted from Durnin and Womersley (1974) (category

1 - average (n=11), category 2 - overweight, (n=15), category 3 - obese, (n=26). Participants were fitted with 4 pedometers (two in position 1=midline of thigh (front), one in position 2=midaxiallary line (side), one in position 3=posterior midline of thigh (back) on the right side of their body at waist level. Participants completed a 40 metre walking course at 3 self selected walking paces of (slow, moderate and brisk) on two occasions. Pedometer steps in each position were compared to steps recorded by direct observation and analysed across body composition categories using repeated measures analysis of variance.

Results: Significant main effect for position ($p<0.001$) and the interaction of position with all body composition groupings ($p<0.001$) were found. Multiple comparison analysis identified differences between observed counts and counts recorded from the pedometers in the side position in BMI category 4 ($p<0.001$), waist/hip ratio category 3 ($p<0.001$) and percentage body fat category 3 ($p<0.001$), and from pedometers in the back position in BMI category 2 ($p<0.001$) and waist/hip ratio category 3 ($p<0.001$). Significant correlations ($p<0.001$) were found at all walking speeds (slow $r=0.84$, moderate $r=0.92$, brisk $r=0.96$) for steps recorded from pedometers in position 1 and 2, indicating high inter-instrument reliability. No significant differences ($p>0.05$) were found between test 1 and test 2 across body composition groupings, pedometer position and walking speed.

Conclusions: The Digiwalker electronic pedometer is valid and reliable across variable body composition and walking speeds when worn in the front (manufacturer recommended) position.

PS 60

GLP-I analogues and incretin effect

0779

Plasma ghrelin concentrations in type 1 diabetic patients with chronic autoimmune gastritis

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Background and Aims: Type 1 diabetic patients (DM1) show an increased prevalence of pernicious anemia whose hystological substrate is an atrophic chronic gastritis located in the corpus of the stomach, the main source of ghrelin.

Aims: To evaluate if DM1 patients with chronic autoimmune gastritis (CAG) ($DM1CAG^+$) have lower concentrations of ghrelin compared with patients with DM1 without CAG ($DM1CAG^-$) and a control group, all of them matched for age, sex and body mass index (BMI). In addition, we seek to evaluate the correlation between ghrelin and a biochemical marker of gastric mucosa atrophy (pepsinogen I) and of hypo/achlorhydria (gastrin).

Materials and Methods: 15 $DM1CAG^+$ patients (9 women, 36.6±12.8 years, BMI 24.3±2.8 Kg/m^2); 15 $DM1CAG^-$ (37.4±12.6 years, BMI 24±2.7 Kg/m^2) and 15 control (35.6±13.4 years, BMI 23,1±2,5 Kg/m^2). Pepsinogen I (ELISA), gastrin (RIA), parietal cell antibodies (PCA) (indirect immunofluorescence) and ghrelin (RIA) were determined in all patients. $HbA1_c$ was only measured in DM1. In all 15 $DM1CAG^+$ an esophagogastroduodenoscopy was performed to take biopsy samples of gastric mucosa. Chromogranin A was the antibody used for the immunohistochemical study of gastric neuroendocrine cell proliferation.

Results: The histological samples of biopsed patients showed different grades of gastric mucosa atrophy and in some cases (10/15) neuroendocrine cell hyperplasia. No differences were found in ghrelin concentrations between groups ($DM1CAG^+$:651.1±282.9 pg/mL; $DM1CAG^-$: 515.1±148.2 pg/mL; Control:670.9±224.6 pg/mL).Gastrin concentrations were higher in $DM1CAG^+$ than $DM1CAG^-$ and the control group ($DM1CAG^+$: 469(225–708) pg/mL; $DM1CAG^-$ 26 (22–30) pg/mL; Control 21 (18.5–42) pg/mL; $p<0.001$). Pepsinogen I was lower in $DM1CAG^+$ than in $DM1CAG^-$ and the control ($DM1CAG^+$: 18.52±15.05 μg/L; $DM1CAG^-$: 113.72±32.9 μg/L; Control 125.46±32.65 μg/L; $p<0.001$). No correlation was found between ghrelin and pepsinogen I. Ghrelin correlated positively with gastrin ($r=0.52$; $p=0.006$) in DM1 (with and without ACG). In $DM1CAG^+$, gastrin levels correlated with the presence of hyperplastic neuroendocrine lesions (159.66 pg/mL vs 690.6 pg/mL; $p=0.05$). No differences were found in ghrelin concentrations regardless of the presence or not of hyperplastic neuroendocrine lesions in $DM1CAG^+$ patients.

Conclusion: No differences were found in plasma ghrelin concentrations between $DM1CAG^+$ and other studied groups, thus ghrelin does not seem to be a good marker of gastric mucosa atrophy in patients with DM1 and chronic autoimmune gastritis.

0780

Exploring the safety of substituting exenatide for insulin in patients with type 2 diabetes who were using insulin in combination with oral antidiabetic agents

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Background and Aims: Exenatide is a first in class incretin mimetic indicated for use in the United States with metformin (MET) and/or a sulphonylurea (SU) in patients with type 2 diabetes, but is not a substitute for insulin in insulin-requiring patients. We explored the safety of substituting exenatide (5 μg BID for 4 wks, 10 μg BID thereafter) for insulin in patients with type 2 diabetes who were using insulin in combination with MET, SU, or both. **Materials and Methods**: HbA_{1c}, fasting serum glucose (FSG), C-peptide, and body weight were measured during this 16-wk pilot study; successful maintenance of glycaemic control, after substituting exenatide for insulin, was predefined as an HbA_{1c} increase <0.5%. 49 patients (mean±SD: age 54±8 y, BMI 34±4 kg/m^2, HbA_{1c} 8.1±1.1%, FSG 8.8±2.6 mmol/L, daily insulin 45±30 U, duration on insulin 3±3 y, duration of diabetes 11±7 y) were randomised 2:1 to either substitute exenatide for insulin or remain on their current regimen. Evaluable patients (n=45) completed≥8 wks of the study or discontinued earlier due to loss of glycaemic control; 4 exenatide patients withdrew for reasons precluding assessment of glycaemic control.
Results: 62% of exenatide-treated patients (18/29) were able to maintain glycaemic control (mean±SEM: HbA_{1c} change −0.5±0.7%, p=.003). The majority (14/18) of the exenatide-treated patients that successfully maintained glycemic control were also taking SU or a combination of SU/MET; 4 of 12 patients in the MET only group maintained control. The remaining 38% (11/29) unable to maintain glycaemic control (HbA_{1c} change+1.6±1.5%, p=.001) had elevated FSG levels by Wk 2 (+3.9± 2.7 mmol/L, p=.005) that increased throughout the study; excess hyperglycaemia was observed in 1 patient. In the exenatide group overall, body weight was decreased at endpoint −4.2±3.0 kg (p<0.001) independent of glycaemic response and median C-peptide was increased ~20%. The overall adverse event profile was consistent with previous exenatide trials. In the insulin reference group, mean insulin doses were similar at baseline (53.8 U/d) and endpoint (51.5 U/d). Body weight remained relatively stable (+0.5±1.7 kg) and 81% of insulin-treated patients (13/16) maintained glycaemic control (HbA_{1c} change −0.3±0.6%).
Conclusion: This pilot study suggests that it is feasible for some patients with type 2 diabetes to sustain glycaemic control when substituting exenatide for insulin; however, deterioration in glycaemic control can occur. Background therapy with SU and FSG levels measured after exenatide initiation may predict treatment success. Further studies are warranted to better evaluate insulin-using patients with type 2 diabetes who may benefit from exenatide treatment.

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0781

Exenatide maintained glycaemic control with associated weight reduction over two years in patients with type 2 diabetes

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Background and Aims: Exenatide is an incretin mimetic that improves glycaemic control with associated weight reduction in patients with type 2 diabetes when used in combination with metformin (MET) and/or sulphonylurea (SU).
Materials and Methods: We analysed data from type 2 diabetes patients not achieving glycaemic control with MET and/or SU enrolled in phase 3 trials followed by open-label extensions such that at the time of this analysis, patients had received≥2 years of exposure to exenatide. The effects of twice-daily treatment of exenatide (10 μg BID) on glycaemic control (HbA_{1c} and fasting plasma glucose [FPG]), HOMA-B, body weight, safety, and tolerability were assessed. At the time of this analysis, 283 patients entering the open-label extension (177 M, 106 F; age 57.3±9.7 yr; BMI 33.7±5.5 kg/m^2; mean±SD; baseline HbA_{1c} 8.3±1.0%; baseline FPG 9.7±0.1 mmol/L; mean-SE) had completed 2 years of follow-up.
Results: The 2-year completer population had reductions from baseline HbA_{1c}, FPG, and body weight of −1.1±0.1%, −1.2±0.2 mmol/L, and −2.4±0.2 kg (mean-SE), respectively at Week 30 of the placebo-controlled trial. After 2 years of treatment, changes in glycaemic control were similar, demonstrating sustained reductions in HbA_{1c} and FPG from baseline of −1.1±0.1% (P<0.05) and −1.4±0.2 mmol/L, (P<0.05) (mean±SE), respectively. Weight reduction from baseline after 2 years was progressive at −4.7±0.3 kg, (P<0.05) (mean±SE). After 2 years, 50% and 31% of patients achieved HbA_{1c} of≤7% and≤6.5%, respectively. Similar results were obtained for the ITT population. In a subgroup of the 2-year completer population (patients who received MET or SU, N=112), HOMA-B improved from baseline by 11% (P<0.05) after 2 years of exenatide exposure. Exenatide was generally well-tolerated during 2 years of exposure. The most frequent adverse events were gastrointestinal in nature.

Conclusion: In this open-label extension study, exenatide exerted sustained improvements in glycaemic control and progressive reduction in body weight out to 2 years in patients with type 2 diabetes.

0782

A comparison of exenatide and insulin glargine in patients using a single oral antidiabetic agent

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Background and Aims: Insulin glargine is a common treatment for type 2 diabetes patients when oral medications no longer provide adequate glycaemic control. Exenatide is an incretin mimetic for the treatment of type 2 diabetes inadequately controlled with metformin (MET) and/or a sulfonylurea (SFU).
Materials and Methods: This randomised, two-period, open-label, crossover study compared effects between exenatide (5 μg BID for 4 wks, then 10 μg BID for 12 weeks) and insulin glargine (QD titrated to fasting blood glucose≤5.6 mmol/L). Either treatment was added to ongoing single oral agent therapy (MET 56%; or SFU 44%) during two, 16-wk treatment periods. Patients (mean±SD) (age, 54±9 y; weight, 86±16.4 kg; HbA1c, 8.9±1.1%; fasting glucose, 12.09±.31 mmol/L) continued their oral agent at maximal dose.
Results: Similar reductions in HbA1c from baseline (n=114, completers) were observed during treatment periods with exenatide (−1.43±0.09%) and glargine (−1.41±0.09%). A similar percentage of patients achieved HbA1c≤7% with exenatide (40%) and glargine treatment (41%). HbA1c≤6.5% was achieved by 24% of exenatide-treated patients compared with 14% of glargine-treated patients (P=.056). Both treatments maintained lowered HbA1c from the first treatment period through the second treatment period. Weight reduction during the first treatment period of exenatide (−2.35 kg) was reversed by glargine (+2.3 kg, n=55), and weight gain during the first treatment period by glargine (+0.75 kg) was followed by weight reduction on exenatide (−2.3 kg, n=59). Overall weight change from baseline was significantly different (P<0.001) between exenatide (−1.95 kg) and glargine treatments (+0.35 kg). Both treatments significantly decreased fasting glucose from baseline (exenatide, −3.04±0.23 mmol/L; glargine, −4.17±0.23 mmol/L; P<0.0001, within- and between-treatment groups). Exenatide injections prior to the morning and evening meals significantly decreased 2-hr post-meal glucose excursions (both P<0.001) compared with glargine injections. Combined 2 hr glucose excursions after all 3 meals were also significantly lower in exenatide-treated patients compared with glargine-treated patients (P=.036). Overall efficacy results were similar between the MET- and SFU-treated subgroups, except for greater weight reduction in patients treated with exenatide and MET (−2.97±4.28 kg) compared with patients treated with exenatide and SFU (−0.61±2.86 kg). Hypoglycemia occurred in a greater percentage of patients treated with SFU (30% exenatide, 35% glargine) compared with patients treated with MET (3% exenatide, 17% glargine; between groups, P=.01). The most common adverse events potentially related to study drug were nausea (33%) and headache (8.7%) during exenatide and insulin glargine treatments, respectively.
Conclusion: In patients receiving ongoing treatment with MET or an SFU, while both exenatide and insulin glargine improved HbA1c and significantly decreased fasting glucose, only exenatide significantly reduced body weight and combined 2 hour post-meal glucose excursions.
Supported by Eli Lilly and Company and Amylin Pharmaceuticals, Inc.

0783

Patient reported outcomes in patients with type 2 diabetes treated with exenatide compared to biphasic insulin aspart

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Background and Aims: Although exenatide and biphasic insulin aspart appear to have similar clinical efficacy, there are several differences between the two treatments that could influence outcomes from the patient's perspective. Therefore, the purpose of this study was to conduct a secondary analysis of clinical trial data to examine whether the two drugs were comparable as assessed by patient-reported outcomes.
Materials and Methods: Patients with type 2 diabetes (n=505) who had inadequate glycaemic control despite treatment with a sulphonylurea and metformin were randomised to receive either twice daily exenatide or biphasic insulin aspart during the 52-week, non-inferiority trial. At baseline, mean age was 59±9, BMI mean 30±4, and HbA1c mean 8.6%±1.1%. At baseline and endpoint patients were administered the EuroQol instrument (EQ-5D) and the SF-36 Vitality Scale. Where validated language translations were available, the Diabetes Symptom Checklist-Revised (DSC-R) and the Treatment Flexibility Scale

were administered at baseline and endpoint. Change from baseline to endpoint was compared for per protocol patients within each treatment, and then between treatment groups with analysis of covariance models−controlling for country and baseline scores.

Results: At endpoint, exenatide and biphasic insulin aspart achieved similar HbA1c reductions (−0.98% and −0.88%, respectively). Exenatide-treated patients exhibited statistically significant improvements from baseline to endpoint in the DSC-R (−0.13, p=.0356), the EQ-5D Index Score (0.03, p=.0399), the EQ-5D VAS score (3.39, p=.0009), and SF-36 Vitality Scale (3.89, p=.0019) scores−no significant difference in the Treatment Flexibility Scale was observed for exenatide patients. There were no statistically significant changes from baseline to endpoint in the biphasic insulin aspart treatment group for any of the four patient-reported outcomes. Exenatide-treated patients demonstrated significant improvement from baseline compared to biphasic insulin aspart-treated patients in the DSC-R (−0.13 vs. 0.05, p=.0218), but not in other patient-reported outcome instruments.

Conclusion: Exenatide-treated patients showed improvement from baseline to endpoint in many patient-reported outcomes. In contrast, biphasic insulin aspart-treated patients demonstrated no similar improvement in patient reported outcomes despite similar glycaemic improvements compared with exenatide. These data indicate that exenatide may be associated with improvements in patient reported outcomes and quality of life in patients with type 2 diabetes.

Supported by Eli Lilly and Company

0784

Exenatide treatment for 82 weeks reduced C-reactive protein, HbA_{1C}, and body weight in patients with type 2 diabetes mellitus

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Background and Aims: Cardiovascular disease (CVD) is a major cause of morbidity and mortality in patients with type 2 diabetes mellitus (T2DM). Elevated C-Reactive Protein (CRP), a proinflammatory peptide, is associated with increased risk of CVD. In T2DM patients not achieving glycemic control with MET and/or SFU and enrolled in phase 3 trials followed by open-label extensions, the incretin mimetic exenatide improved glycemic control, diastolic blood pressure, serum HDL-C, and serum triglycerides; reduced body weight, and was generally well tolerated with mild-to-moderate nausea the most common adverse event. We investigated whether there were CRP changes after 82 wks of exenatide treatment in a subset of patients with evaluable serum samples (66% M, age 57± 10 y, wt 97±20 kg, BMI 33±5 kg/m^2, HbA_{1c} 8.2±1.0% [mean±SD]).

Materials and Methods: A *post hoc* interim analysis of serum high-sensitivity CRP was performed (n=178). CRP data was log transformed for correlation and regression analyses due to non-normal data distribution. Patients with CRP≥10 mg/L at any visit were excluded from the analysis to avoid confounding the results by including patients with generalized inflammatory disease.

Results: Patients completing 82 wks on exenatide with evaluable serum samples had reductions in HbA_{1c} (−1.2±0.1%) and body weight (−4.5±0.4 kg) from baseline (mean±SE). Median serum CRP concentrations decreased significantly by −1.35 mg/L from baseline median 3.21 mg/L (p<0.0001). In multiple regression analysis adjusting for baseline CRP, both HbA_{1c} change and weight change were significantly associated with Week 82 CRP values (p=0.0244 and p<0.0001, respectively). Subgroup analysis stratifying by baseline CRP≤3 mg/L (low/moderate CVD risk) or>3 mg/L (high CVD risk) revealed a marginal decrease in median CRP for the low risk group (−0.43 mg/L; −22%; p=0.0617), contrasted with a significant reduction in median CRP of −2.25 mg/L (−50%; p<0.0001) for the high risk group.

Conclusion: Exenatide treatment for 82 weeks was associated with a reduction in CRP, and this was correlated with reduced HbA_{1c} and body weight in T2DM patients. The greatest CRP decrease occurred in the high CVD risk group, suggesting further studies are warranted into whether exenatide can improve CVD risk in this patient population.

0785

Safety and efficacy of exenatide over 16 weeks in patients with type 2 diabetes mellitus using a thiazolidinedione with or without metformin

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Background and Aims: Exenatide, an incretin mimetic for the treatment of type 2 diabetes mellitus (T2DM), mimics

several glucose-lowering actions of GLP-1 including glucose-mediated insulin secretion. Adjunctive therapy of exenatide and a TZD with or without metformin may reduce glucose levels by targeting the central defects of T2DM: beta cell dysfunction and insulin resistance.

Materials and Methods: This study was a randomised, placebo-controlled, parallel, double-blind trial in 233 patients with elevated HbA_{1c} (range: 7.1–10.0%) in spite of therapy with a TZD alone (20%) or a TZD with metformin (80%). Patients (129M, 56±10 y, BMI 34±5 kg/m^2, HbA_{1c} 7.9±0.9% [mean±SD]) received subcutaneous injections of placebo or exenatide BID for 16 weeks. Patients that received exenatide received 5 μg BID for 4 weeks followed by 10 μg BID for 12 weeks. Endpoint measures included HbA_{1c}, fasting serum glucose (FSG), body weight, 7-point self-monitored blood glucose (SMBG), safety, and tolerability.

Results: For exenatide, 71% of patients completed the study versus 86% for placebo. Exenatide decreased mean HbA_{1c} by −0.8±0.9% from baseline (treatment effect, −0.9%, p<0.0001). With exenatide, 62% of patients achieved HbA_{1c}≤7% versus 16% with placebo (p<0.0001); 29% achieved HbA_{1c}≤6.5% with exenatide versus 8% with placebo (p=0.0002). Mean FSG for patients who received exenatide was −1.5 mmol/L lower than placebo (p<0.0001). Exenatide reduced mean body weight from baseline versus placebo (−1.5±3.1 kg vs. −0.2±2.5 kg, respectively, p<0.001). Mean daily 2-h SMBG postprandial excursions for exenatide were lower than baseline (−1.4±1.4 mmol/L, p<0.0001) with greatest differences after breakfast (−1.9±2.9 mmol/L, p<0.0001) and dinner (−1.9±2.5 mmol/L, p<0.0001). Placebo-treated patients showed no significant reductions in postprandial glucose excursions from baseline at any meal but demonstrated a reduction in the daily mean glucose concentration (p=0.0408). Exenatide reduced the proinsulin/insulin ratio over placebo (p=0.0337) and increased HOMA-B versus placebo (p=0.0032). No statistically significant difference in HOMA-S existed between groups. The most frequent adverse event was nausea (40% exenatide vs. 15% placebo); there was no significant difference in incidence of hypoglycaemia.

Conclusion: These findings support the potential use of exenatide as adjunctive therapy for patients with T2DM who inadequately control glucose levels with a TZD alone or in combination with metformin.

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0786

Liraglutide significantly improves glycaemic control, and lowers body weight without risk of either major or minor hypoglycaemic episodes in subjects with type 2 diabetes

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Background and Aims: The objective of this 14-week study was to assess the efficacy and safety of liraglutide, a once-daily GLP-1 analogue.

Materials and Methods: 165 subjects with type 2 diabetes with an HbA_{1c} at randomization of 8.1–8.5% were studied. Subjects treated with diet (19%) or single oral antidiabetic agents (after 4 weeks washout) were randomized to one of three once-daily doses of liraglutide (0.65 mg, 1.25 mg or 1.9 mg) or placebo (1:1:1:1).

Results: Significant improvement in HbA_{1c} was achieved in all actively treated groups vs. placebo (p<0.0001), with an estimated difference vs. placebo of −1.74% at the highest dose level (last observation carried forward - LOCF). The fraction of subjects achieving an HbA_{1c} level≤7.0% was between 0.43 and 0.50 in the three liraglutide arms versus 0.08 in the placebo group. Improvement in glycemic control was achieved without any major or minor hypoglycemic episodes. Dose dependent weight reduction was achieved. In the high-dose liraglutide treatment group, estimated change in body weight was −2.99 kg from baseline and −1.21 kg compared to placebo (p=0.039) (LOCF). Liraglutide was well tolerated; four subjects withdrew due to adverse events in the three liraglutide treated groups vs. three in the placebo treated group. Main adverse events were from the gastrointestinal (GI) system; diarrhea was the most frequent, with an incidence of 19.5% and 12.5% in the high-dose liraglutide group and in the placebo group, respectively. In the high-dose group 10% of the subjects experienced nausea. The frequency of GI events decreased over time.

Conclusion: Liraglutide used as monotherapy significantly improved glycemic control without risk of major or minor hypoglycemia, was well tolerated, and lowered body weight in subjects with type 2 diabetes.

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0787

Cost-effectiveness of exenatide versus insulin glargine for the treatment of type 2 diabetes in a UK setting: a long-term health economic study

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Background and Aims: A recent randomised open-label, parallel group, clinical trial in 549 patients with type 2 diabetes unable to achieve adequate glycaemic control using sulphonylurea and metformin was performed to compare exenatide to insulin glargine. After 26-weeks, exenatide and insulin glargine were associated with a decrease in HbA1c values (−0.99% vs −1.07%). The most notable difference was a reduction in body weight in patients treated with exenatide (−2.32 kgs) compared with an increase in body weight for the insulin glargine treatment arm (+1.75 kgs). Although exenatide is currently available in the US, it has not yet been approved in the UK. The aim of the present analysis was to determine in the UK, over a range of prices, the incremental cost-effectiveness ratio (ICER) for exenatide compared to insulin glargine.
Materials and Methods: A previously published model of type 2 diabetes was used to project the clinical and economic outcomes from the clinical trial over patient lifetimes. The model simulates disease progression by combining 15 Markov-based sub-models to determine the costs and clinical outcomes associated with the occurrence of disease-related complications. Baseline cohort characteristics (mean age 58.9 yrs; 55.7% male; mean HbA1c 8.21%; duration of diabetes 9.56 yrs) and treatment effects were taken from the clinical trial. The study assumed the perspective of the UK national health service payer using treatment costs presented in 2004 British Pounds(£). As no price is currently available for exenatide in the UK, the US price ($5.75 per day, approximately £3.28) was used as a point estimate, with the price reduced in increments of 20% in exploratory scenarios. Medication costs and utilities/disutilities based on weight loss and weight gain and other significant product attribute differences were included in the model. A discount rate of 3.5% was applied to costs and clinical outcomes.
Results: Exenatide was associated with a higher life expectancy (10.05 vs 9.99 years) and quality-adjusted life expectancy (7.01 vs 6.00 QALYs) compared to insulin glargine, primarily attributed to a reduction in CV complications, as determined by the CORE Diabetes Model that uses projective risk equations. Lifetime direct medical costs were higher in the exenatide group for prices at 40 to 100% of the US price with ICERs between £1124 and £8722 per QALY gained, compared to insulin glargine.
Conclusion: Using the CORE Diabetes Model to simulate long-term outcomes, treatment with exenatide was associated with a higher life expectancy and an improvement in quality-adjusted life expectancy compared to insulin glargine. Using the US daily price, exenatide was estimated to have an ICER of £8722 per QALY gained compared to insulin glargine, well below the thresholds commonly referred to as representing good value for money in the UK.

	Exenatide medical costs	Insulin Glargine medical costs	Difference in costs	ICER (Costs per QALY)
100% of US price	£31,877	£23,073	£8,804	£8,722
80% of US price	£29,320	£23,073	£6,247	£6,189
60% of US price	£26,764	£23,073	£3,691	£3,657
40% of US price	£24,208	£23,073	£1,135	£1,124
20% of US price	£21,651	£23,073	−£1,422	Dominant

Supported by Eli Lilly and Company

PS 61

DPP-4 inhibitors

0788

Vildagliptin displays slow tight-binding to dipeptidyl peptidase (DPP)-4, but not DPP-8 or DPP-9

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Background and Aims: Vildagliptin [(S)1-[(3-hydroxy-adamant-1-ylamino)acetyl]pyrrolidine-2-carbonitrile] is an inhibitor of DPP-4 in late stage clinical development for treatment of patients with type 2 diabetes. Vildagliptin potently inhibits DPP-4 with an IC_{50} of approximately 3 nM. The chirality of the nitrile functionality of vildagliptin (which is the S-enantiomer) was found to be critical to its inhibitory action, as the IC_{50} of the R-antipode for inhibition of DPP-4 was>1100-fold higher than that of vildagliptin. Vildagliptin is highly selective for DPP-4 despite the similarity of substrate specificities among many physiologically important proteases. When tested at concentrations up to 100 microM, vildagliptin elicited little or no inhibition of human aminopeptidase P, human leukocyte elastase, porcine trypsin, rabbit angiotensin converting enzyme or rat neutral

endopeptidase 24.11. Vildagliptin was found to be a weak inhibitor of human post-proline cleaving enzyme, with an IC_{50} of 206 microM. The current study evaluates the selectivity and binding characteristics of vildagliptin for four closely related serine proteases: DPP-2, DPP-8, DPP-9 and fibroblast activation protein alpha (FAP alpha; seperase).
Materials and Methods: The kinetics of inhibition of Caco-2 DPP-4 and recombinant human DPP-2, DPP-4, DPP-8, DPP-9 and FAP alpha by vildagliptin were evaluated to establish the K_i and the half-lives of dissociation of the enzyme-inhibitor complexes after 100-fold dilution. Kinetic measurements were derived from progression curves of AMC formation from the substrate H-Gly-Pro-AMC at 25 degrees C. Furthermore, the potential modification of vildagliptin by DPP-4 was assessed by LC/MS analysis of incubation reactions.
Results: Kinetic analysis of vildagliptin-induced inhibition of DPP-4 from Caco-2 cells indicated that, although vildagliptin is a substrate analog that displays apparent single-site binding behavior, it does not exhibit a simple competitive inhibitor pattern. Rather, the kinetics of vildagliptin-induced inhibition of DPP-4 suggest a slow tight-binding mechanism. The K_is of vildagliptin for DPP-2, DPP-4, DPP-8, DPP-9 and FAP alpha were>20,000, 3, 810, 95, and 9,900 nM, respectively. Further, the very slow dissociation of the vildagliptin/DPP-4 complex ($t_{1/2}$=55 min) is in contrast with the very rapid dissociation of vildagliptin from DPP-8 or DPP-9 (<10 sec). The high potency and slow dissociation kinetics of vildagliptin for DPP-4 are due to the formation of a transient high-affinity complex that represents a transitory imidate intermediate which leads to cleavage of the carbon-nitrogen bond in the nitrile moiety of the compound, generating an inactive carboxylic acid byproduct.
Conclusion: Vildagliptin is a substrate for DPP-4 that displays slow tight-binding kinetics with very slow dissociation rates. This contrasts with vildagliptin's rapid dissociation kinetics for the related proteases DPP-8 and DPP-9. These differences in dissociation rates for DPP-4 relative to DPP-8 and DPP-9 infer greater selectivity among these related proteases than the simple ratio of K_i. would suggest, reducing the potential of vildagliptin to inhibit these enzymes at pharmacologic doses.

0789

Vildagliptin decreases HbA_{1c} after 12 week treatment in Japanese patients with type 2 diabetes

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Background and Aims: Vildagliptin is a potent and selective DPP-4 inhibitor that improves glycemic control by increasing pancreatic α- and β-cell responsiveness to glucose. The purpose of the present study was to assess the efficacy and tolerability of vildagliptin monotherapy in Japanese patients with type 2 diabetes (T2DM)
Methods: This was a 12-wk, randomized, double-blind, placebo (PBO)-controlled study of vildagliptin (10 mg bid, 25 mg bid or 50 mg bid) in 219 drug-naïve Japanese patients with T2DM. A standard meal test (SMT, Japanese meal, kcal=470, CHO=73%, protein=12%, fat=15%) was conducted at baseline (BL) and Wk 12 in a subset of patients.
Results: In the randomized population, the mean±SD age and BMI were 59±8.4 y and 24.4±2.8 kg/m^2, respectively. As reported below, vildagliptin decreased HbA_{1c} and FPG in a dose-related manner whereas glycemic control deteriorated slightly in patients receiving PBO.

Mean±SE	HbA_{1c} (%) Baseline	Δ	FPG (mM) Baseline	Δ
PBO (n=72)	7.4±0.8	0.3±0.1	9.0±1.7	0.1±0.2
Vildagliptin 10 mg bid (n=71)	7.4±0.8	−0.5±0.1*	8.9±1.9	−0.6±0.2*
Vildagliptin 25 mg bid (n=72)	7.4±0.9	−0.7±0.1*	9.0±1.8	−0.8±0.2*
Vildagliptin 50 mg bid (n=76)	7.4±0.8	−0.9±0.1*	8.9±1.8	−1.4±0.2*

*P<0.001 vs PBO

During the SMT, peak prandial GLP-1(7-36) increased about 2-fold at Wk 12 relative to BL in vildagliptin-treated patients. In PBO-treated patients, the 2-hr prandial glucose level increased by 0.2 mM and it decreased by 3.5, 3.2 and 3.4 mM in patients receiving vildagliptin 10, 25 and 50 mg bid respectively (p<0.001 for all vs. PBO). Adverse events (AEs) were reported by 74% of patients receiving PBO and by 62% in each of the vildagliptin treatment groups. There was a similar incidence of gastrointestinal AEs in all groups. Hypoglycemia was reported by 1.4, 4.2, 0 and 2.6% of patients receiving PBO and vildagliptin 10, 25, 50 mg bid, respectively.
Conclusions: In Japanese patients with T2DM, 12-wk treatment with vildagliptin significantly decreases HbA_{1c} in a clinically meaningful and dose-dependent manner. This reflects decreases in both FPG and postprandial glucose. Vildagliptin is an effective and well-tolerated approach to achieving glycemic control in Japanese patients with T2DM.
Supported by Novartis

0790

Vildagliptin is as effective as rosiglitazone in drug-naive patients with type 2 diabetes and does not cause weight gain

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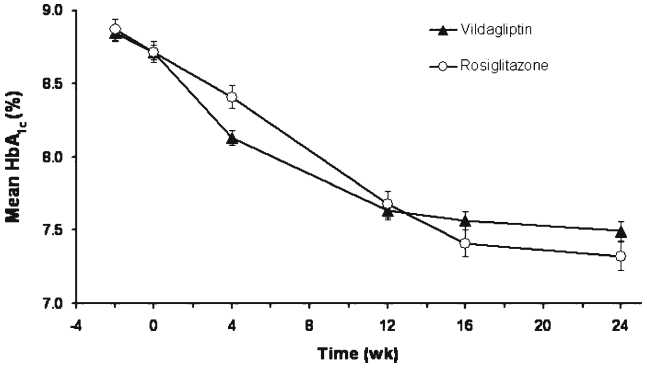
Background and Aims: Vildagliptin is a potent and specific DPP-4 inhibitor that improves glycemic control by increasing pancreatic α- and β-cell responsiveness to glucose. The purpose of this study was to compare the efficacy and tolerability of vildagliptin and rosiglitazone (Rosi) in drug-naïve patients (pts) with type 2 diabetes (T2DM).
Methods: This was a 24-wk, multicenter, randomized, double-blind, parallel group, active-controlled study comparing vildagliptin 50 mg bid (n=459) to Rosi 8 mg qd (n=238) in drug-naïve pts with T2DM. The primary endpoint was the between-group difference and associated 95% confidence interval in the adjusted mean change (AMΔ) in HbA_{1c} from baseline (BL) to Wk 24 or study endpoint.
Results: The two groups were well balanced at BL. Mean age=54.4 y; BMI=32.4 kg/m^2; duration of diabetes=2.4 y and HbA_{1c}=8.7%. As shown below, the two agents decreased HbA_{1c} progressively and to a similar extent, although the onset of action of vildagliptin appeared to be more rapid than that of Rosi. Thus, the mean change at Wk 4 was −0.6±0.0% in pts receiving vildagliptin and −0.3±0.0% in those receiving Rosi. The AMΔ HbA_{1c} from BL to endpoint in pts receiving vildagliptin 50 mg bid was −1.1±0.1%, and that in Rosi-treated pts was −1.3±0.1%. The study established non-inferiority of vildagliptin 50 mg bid to Rosi 8 mg qd.



In pts with BL HbA_{1c}>9.0%, the ΔHbA_{1c} was −1.8±0.1% in the vildagliptin group (n=166) and −1.9±0.2% in the Rosi group (n=88). In non-obese pts (BMI <30 kg/m^2) vildagliptin appeared to be somewhat more effective (ΔHbA_{1c}=−1.3±0.1%, n=184) than Rosi (Δ HbA_{1c}= −1.1±0.2%, n=83). Relative to Rosi, vildagliptin improved the lipid profile, producing significant decreases in triglycerides (−9%, P=0.010), total (−14%, P<0.001), LDL (−16%, P<0.001), and non-HDL cholesterol (−16%, P<0.001), but a smaller increase in HDL-cholesterol (+3.9 vs +8.9%, −5%, P=0.003). Vildagliptin did not affect body weight (BW, AMΔ=−0.3±0.2 kg) but BW increased in Rosi-treated pts (AMΔ=+1.6±0.3 kg, P<0.001 vs vildagliptin). The overall incidence of side effects was 61.4% in pts receiving vildagliptin and 64.0% in those receiving Rosi. The incidence of edema-related events was greater with Rosi (4.9%) than with vildagliptin (2.5%). One patient in each group experienced one mild hypoglycemic episode.
Conclusions: Vildagliptin 50 mg bid 1) is efficacious and well-tolerated in drug-naïve pts with T2DM; 2) is non-inferior to Rosi 8 mg qd; 3) does not affect BW and is associated with less edema and an improved lipid profile relative to Rosi 8 mg qd.
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0791

Efficacy of vildagliptin in drug-naive patients with type 2 diabetes

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Background and Aims: Vildagliptin is a DPP-4 inhibitor shown in Phase 2 studies to improve glycemic control by increasing pancreatic islet responsiveness to glucose. The purpose of the present trial was to assess the efficacy and tolerability of three vildagliptin monotherapy regimens in drug-naïve patients (pts) with type 2 diabetes (T2DM).
Materials and Methods: This was a 24-wk, multicenter, randomized, double-blind, parallel-group study of vildagliptin 50 mg qd (n=104), 50 mg bid (n=90), 100 mg qd (n=92) and placebo (PBO, n=94) in drug-naïve pts with HbA_{1c} of 7.5–10%. The primary endpoint was the between-group difference in the adjusted mean change (AMΔ) in HbA_{1c} from baseline (BL) to Wk 24 or study endpoint, comparing each active treatment to PBO by ANCOVA.
Results: The four treatment groups were well-balanced. At BL, mean age=53.6 y, BMI=32.8 kg/m^2, disease duration=2.0 y and HbA_{1c}=8.4%. The between-group difference in AMΔ HbA_{1c} was −0.5±0.1% (P=0.006), −0.5±0.1% (P=0.006) and −0.6±0.1% (P=0.001 vs PBO) in pts receiving vildagliptin 50 mg qd, 50 mg bid and 100 mg qd respectively. An unusual study effect of PBO

was observed (AMΔ=−0.3±0.1%), largely driven by 16% of patients who were enrolled within 2 wks of diagnosis (Dx). In this small newly-diagnosed cohort, HbA_{1c} decreased by more than 1% in all treatment groups, with no significant difference between vildagliptin and PBO. However, in pts diagnosed>2 wks prior to enrollment, vildagliptin elicited significant decreases in HbA_{1c} and little PBO effect was observed. Since clinical trials have traditionally recruited patients with a Dx>3 months, and physicians often employ this time frame prior to initiating pharmacotherapy (presumably for a trial of diet and exercise), data were evaluated using this criterion. In patients who were diagnosed>3 mos before enrollment (60%) there was a small increase in HbA_{1c} in the PBO group (AMΔ=0.2±0.2%) and the between-group difference in AMΔ HbA_{1c} was −0.8±0.2% in the 50 mg qd group (P<0.001), −0.7±0.2% in the 50 bid group (P=0.003) and −0.9±0.2% in pts receiving 100 mg qd (P<0.001 vs PBO). Overall, vildagliptin was well-tolerated. Mild hypoglycemia was reported by 1.2%, 0%, 0.6% and 0% of pts randomized to 50 mg qd, 50 mg bid, 100 mg qd and PBO, respectively. There was a modest decrease in mean BW in each treatment group, ranging from an AMΔ body weight of −0.3 kg in the 50 mg bid group to −1.8 kg in the 50 mg qd group.

Conclusions: 1) Vildagliptin produces clinically meaningful and statistically significant decreases in HbA_{1c} in drug-naïve patients; 2) Vildagliptin monotherapy is well tolerated; it does not cause weight gain and is associated with a low risk of hypoglycemia.

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0792

WITHDRAWN

0793

Vildagliptin added to metformin improves glycaemic control by decreasing fasting and postprandial glucose in patients with type 2 diabetes

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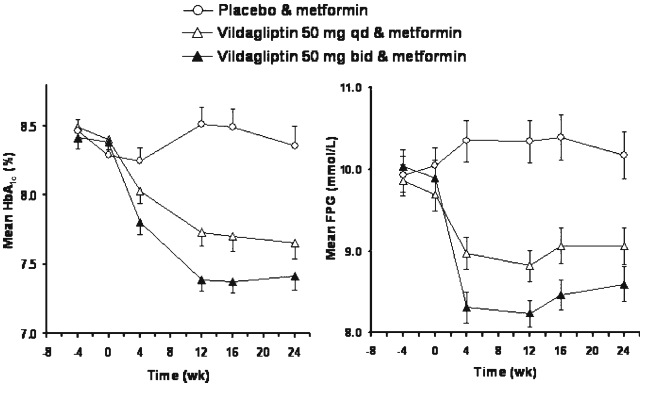
Background and Aims: Vildagliptin is a potent and selective DPP-4 inhibitor that improves glucose metabolism by increasing α- and β-cell responsiveness to glucose. The purpose of this study was to assess the efficacy and tolerability of vildagliptin when added to metformin treatment in patients (pts) with type 2 diabetes (T2DM).

Materials and Methods: This was a 24-wk, multicenter, double-blind, randomized, parallel group study comparing vildagliptin (50 mg qd or bid) to placebo (PBO) in 416 patients with T2DM continuing a previous stable metformin regimen (≥1500 mg/d). The primary endpoint was the between-group difference in the adjusted mean change (AMΔ) in HbA_{1c} from baseline (BL) to Wk 24 or study endpoint, comparing both active treatments to PBO by ANCOVA.

Results: The 3 treatment groups were well-balanced. At BL mean age=54.2±9.8 y, BMI=32.8±5.5 kg/m^2, disease duration=6.2±5.2 y, HbA_{1c}=8.4±1.0%, metformin dose=2109±315 mg/d and duration of metformin use=17.2±21.3 mo. As shown below, in pts receiving vildagliptin, the mean HbA_{1c} and FPG decreased rapidly and progressively during the first 12 wks of treatment, and appeared to reach a steady state at Wk 16. The between-group difference in the AMΔ HbA_{1c} was −0.7±0.1% in pts receiving vildagliptin 50 mg qd (P<0.001 vs PBO) and −1.1±0.1% in pts receiving 50 mg bid (P<0.001 vs PBO). The proportion of pts reaching target HbA_{1c} <7.0% was 3-4-fold higher in the vildagliptin groups than in the PBO group (P<0.001). The between-group difference in the AMΔ in FPG was −0.8±0.3 mM (P=0.003 vs PBO) and −1.7±0.3 mM (P<0.001 vs PBO) in pts receiving vildagliptin 50 mg qd and 50 mg bid, respectively.



A standard meal test was performed in pts volunteering to participate (~40%, ~55 pts/group). Relative to PBO, the 2-h postprandial glucose (PPG) level was significantly decreased in vildagliptin-treated pts. The between-group difference in AMΔ PPG was −1.9±0.6 mM in the 50 mg qd group (P=0.001 vs PBO) and −2.3±0.6 mM in the 50 mg bid group (P<0.001 vs PBO). This was associated with an improvement in several measures of β-cell function. There was no weight gain in the vildagliptin groups and the incidence of side effects did not differ between groups (63.3, 65.0 and 63.5% of pts randomized to vildagliptin

50 mg qd, 50 mg bid and PBO, respectively). Notably, 9.6%, 14.8% and 18.2% of pts receiving vildagliptin 50 mg qd, 50 mg bid and PBO experienced≥1 gastrointestinal side effect and the incidence in the vildagliptin 50 mg qd group was significantly lower (P=0.022) than in the PBO group. One hypoglycemic event occurred in each group.
Conclusions: Vildagliptin is well-tolerated and produces clinically meaningful, dose-related decreases in FPG, PPG and HbA_{1c} in metformin-treated patients with T2DM.
Supported by Novartis

0794

Efficacy and safety of sitagliptin over 18 weeks in patients with type 2 diabetes

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Background and Aims: Sitagliptin is a new, orally-active, potent and selective dipeptidyl peptidase-4 (DPP-4) inhibitor currently in development for the treatment of type 2 diabetes. Sitagliptin acts by blocking DPP-4-mediated inactivation of active incretin hormones that are released into circulation following ingestion of a meal. Incretins increase insulin release and lower glucagon secretion in a glucose-dependent manner, leading to a suppression of post-meal increases in glucose concentration and a reduction in fasting glucose concentrations. In addition to improving glycemic control, sitagliptin treatment may improve pancreatic β-cell function and may also lead to β-cell preservation. The present randomized, double-blind, placebo(PBO)-controlled, Phase III study assessed the efficacy and safety of once-daily sitagliptin in patients with type 2 diabetes.
Material and methods: After a washout period for patients on an antihyperglycemic agent, patients with HbA_{1c}≥7% and≤10% entered a 2-week single-blind run-in period, at the completion of which, 521 patients ages 27 to 76 yrs were randomized in a 1:2:2 ratio to PBO, sitagliptin 100 mg q.d., or 200 mg q.d., for 18 weeks. Patients not meeting pre-specified glycemic thresholds were provided rescue therapy (metformin) until study completion. Mean baseline HbA_{1c} for the study population was 8.1%. The efficacy analysis was based on an all-patients-treated population using an ANCOVA and excluded data obtained after glycemic rescue.
Results: After 18 weeks, HbA_{1c} was significantly reduced with sitagliptin 100 mg and 200 mg compared with PBO (mean PBO-subtracted change from baseline in HbA_{1c}: −0.60% and−0.48%, respectively). Patients with higher baseline HbA_{1c} (≥9%) experienced greater PBO-subtracted reductions in HbA_{1c} with sitagliptin treatment (−1.20% for 100 mg and −1.04% for 200 mg) than those with baseline HbA_{1c} <8% (−0.44% and −0.33%, respectively) or 8% to 8.9% (−0.61% and −0.39%, respectively). Sitagliptin significantly decreased fasting plasma glucose (FPG) relative to PBO after 18 weeks (mean PBO-subtracted change from baseline in FPG: −1.09 mmol/L for 100 mg and −0.94 mmol/L for 200 mg). In a subset of patients who completed a meal tolerance test (n=150), 2-hour post-meal glucose (PPG) was significantly reduced with with both doses of sitagliptin compared with PBO (mean PBO-subtracted change from baseline in 2-hour PPG: −2.57 mmol/L and −2.92 mmol/L, respectively). There were no significant differences between the two sitagliptin doses for their effects on HbA_{1c}, FPG, or PPG. HOMA-β was significantly increased for both sitagliptin doses, and the fasting proinsulin/insulin ratio was significantly decreased with sitagliptin 100 mg. Sitagliptin was well tolerated with no significant increased incidence of hypoglycemia or gastrointestinal adverse experiences with sitagliptin compared to PBO. Mean body weight was similarly reduced with sitagliptin 100 mg (−0.6 kg), 200 mg (−0.2 kg), and PBO (−0.7 kg) after 18 weeks.
Conclusion: In this study, sitagliptin monotherapy improved glycemic control as well as measures of β-cell function and was well-tolerated over 18 weeks in patients with type 2 diabetes.
Supported by Merck & Co. Inc.

0795

Sitagliptin, a DPP-4 inhibitor: an overview of the pharmacokinetic (PK) profile and the propensity for drug-drug interactions (DDI)

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Background and Aims: Patients with type 2 diabetes are usually on polypharmacy to treat hyperglycemia and other co-mordib conditions. The PK profile and propensity for DDI were extensively examined during the development of sitagliptin.
Materials: Twenty-seven Phase I studies that characterized the PK and pharmacodynamic characteristics and initial safety and tolerability of sitagliptin were conducted in healthy subjects and patients with type 2 diabetes.
Results: Sitagliptin exposure (AUC) increases dose proportionally with increasing dose. At 100-mg dose, apparent terminal elimination half-life is ~10 to 12 hrs. Steady-state

is generally reached by 3 days and accumulation is slight (~14% following 100-mg dose). There is no effect of food on PK. Sitagliptin is well absorbed, with an absolute bioavailability of 87%. Sitagliptin is primarily renally eliminated as unchanged drug (~75% of oral dose excreted unchanged in urine), with metabolism playing only a minor role (primarily cytochrome P-450 (CYP) 3A4 mediated). Given a renal clearance of ~350 mL/min, active tubular secretion appears to be involved in the elimination of sitagliptin, but it is not definitively known which transporters are involved in this process *in vivo*. In vitro studies suggest that the organic anionic transporter-3 may play a role, but organic cationic transporters (OCT) and peptide transporter-1 transporters do not transport sitagliptin. Sitagliptin is a human p-glycoprotein (Pgp) substrate, and thus Pgp may influence the absorption and elimination of sitagliptin. However, the observed modest effects of supratherapuetic doses of cyclosporine A (AUC increased ~29%), a potent probe Pgp inhibitor, on sitagliptin PK suggest that the potential for clinically meaningful Pgp-mediated drug interactions is limited. In vitro, sitagliptin is not an inhibitor of CYP enzymes and is not an inducer of CYP3A4. In clinical studies, sitagliptin did not meaningfully alter the PK of metformin, rosiglitazone, glyburide, simvastatin, warfarin, or oral contraceptives providing further *in vivo* evidence for a low propensity for perpetrating drug interactions with substrates of hOCT, CYP3A4, CYP2C8, and CYP2C9. Multiple doses of sitagliptin 100 and 200 mg slightly increased plasma immunoreactive digoxin concentrations ~(11% and ~18% increase in AUC, respectively), however, these increases were not considered likely to be clinically meaningful. Sitagliptin concentrations are not meaningfully altered by metformin. Sitagliptin exposure is increased by approximately 2.3, 3.8, and 4.5-fold relative to healthy subjects for patients with moderate renal insufficiency [RI] (creatinine clearance [CrCl]=30 to <50 mL/min), severe RI (CrCl <30 mL/min), and ESRD (on dialysis), respectively. At doses of 100 mg or higher, weighted average plasma DPP-4 inhibition was 80% or greater and produced 2 to 3-fold increases in active glucagon-like peptide-1 and glucose-dependent insulinotropic peptide levels over 24 hours postdose. Sitagliptin was generally well-tolerated in the various patient cohorts studied. In a population PK analysis of Phase I and IIb studies, RI was the only factor found to have a potentially clinically relevant effect on PK. Concomitant medications (n=83) were also screened for potential effects on sitagliptin PK; no clinically relevant DDIs were detected.

Conclusion: Sitagliptin has the PK profile consistent with once-daily dosing and a low propensity to be involved in DDI as either a perpetrator or a victim.

Supported by Merck & Co. Inc.

0796

Insulinotropic actions of nateglinide are accompanied by inhibition of dipeptidyl peptidase-IV activity

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Background and Aims: Nateglinide restores early phase insulin secretion to feeding and reduces postprandial hyperglycaemia in type 2 diabetes. Gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1(7–36)amide (GLP-1), natural mediators of enhanced postprandial insulin secretion, are rapidly cleaved by dipeptidyl peptidase (DPP-IV) resulting in rapid clearance from the circulation. This study examined the effect of nateglinide on DPP-IV activity and incretin hormone degradation in type 2 diabetic patients.

Materials and Methods: DPP-IV activity in type 2 diabetic subjects (n=10, age 57.1±1.9 y, BMI 34.7±2.6 kg/m^2, fasting glucose 9.3±1.2 mmol/l) was measured following administration of oral nateglinide (120 mg) or placebo at time t −10 min followed by a 75 g oral glucose load at t0 min in a randomised cross-over design. Other parameters measured included HbA_{1c}, plasma glucose, insulin and C-peptide concentrations. Effects of nateglinide on GIP degradation were further investigated in pooled human plasma using reverse-phase HPLC and electrospray ionization mass spectrometry.

Results: The mean activity of DPP-IV in the placebo group (HbA_{1c} 7.3±0.6%, glucose 9.6±1.4 mmol/l, insulin 99.8±17.7 pmol/l) and in the nateglinide group (HbA_{1c} 7.9±0.9%, glucose 9.1±1.0 mmol/l, insulin 108.9±19.8 pmol/l) prior to treatment was 27.4±0.7 and 29.6±2.3 nmol/ml/min, respectively. Following nateglinide, DPP-IV activity was reduced after 10 min (19.9±1.6 nmol/ml/min, $p<0.05$), reaching a minimum of 1.9±0.1 nmol/ml/min at 120 min ($p<0.001$). Mean DPP-IV activity in the placebo group was 22.5±0.6 nmol/ml/min and decreased to a minimum at 40 min (18.9±0.6 nmol/ml/min) and 120 min (18.3±0.4 nmol/ml/min). Circulating insulin and C-peptide levels increased in the nateglinide group to 637.6±163.9 pmol/l ($p<0.05$) and 11.8±1.4 mg/l ($p<0.01$), respectively, compared to placebo. Plasma glucose reached 18.2±1.7 mmol/l and 16.7±1.7 mmol/l at 90 min in the placebo and nateglinide groups, respectively ($p<0.001$). Percentage intact GIP(1–42) remaining in nateglinide-treated type 2 diabetic plasma at 2, 4 and 8 h incubations was 93±2.2%, 83±0.0% ($p<0.05$) and 64±1.0% ($p<0.01$), and was higher compared with placebo (91±0.4%, 72±1.4% ($p<0.05$) and 38±0.7% ($p<0.01$)), respectively. Nateglinide

inhibited the degradation of GIP(1–42) at 2, 4 and 8 h in human plasma, at concentrations of 62.5 μM by 79.1±1.6% ($p<0.05$), 64.7±1.5% ($p<0.05$), 48.0±3.1% ($p<0.001$), 125 μM by 81.5±2.2% ($p<0.05$), 73.0±1.7% ($p<0.01$), 64.0±2.0% ($p<0.001$) and 250 μM by 100±0% ($p<0.001$), 100±0% ($p<0.001$), 70.0±1.7% ($p<0.001$), respectively.
Conclusion: DPP-IV activity was inhibited by oral nateglinide in type 2 diabetic subjects with reduced degradation of GIP(1–42) to its inactive N-terminally truncated form, GIP (3–42). This action may play a role in the activity of prandial insulin-releaser agents.

PS 62

Oral agents: new drugs and new combinations

0797

Metformin gum: an alternate method for delivery of biguanides

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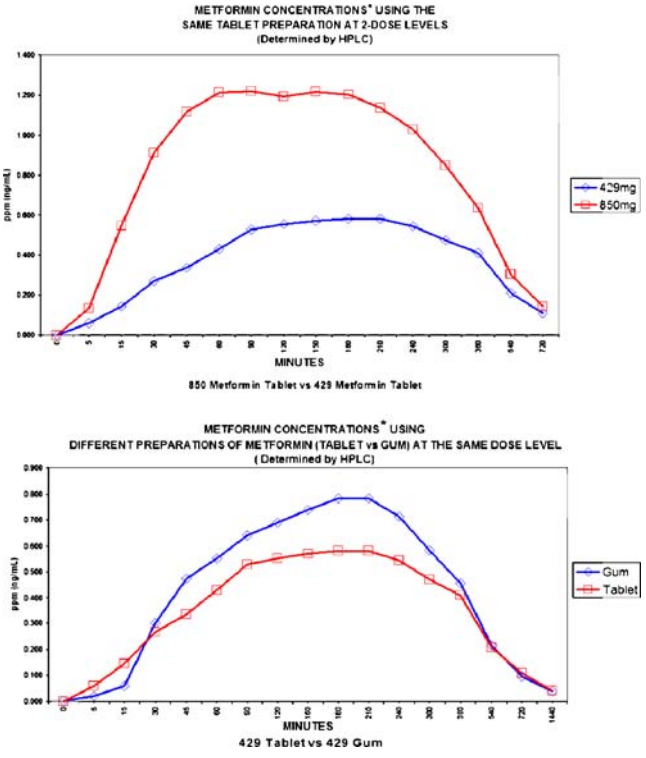
Background and Aims: Metformin (Met) is widely used in the treatment of Diabetes Mellitus due to its efficacy, comparable to that of sulfonylureas, but devoid of side effects such as weight gain and hypoglycemia. Weight loss seen with the use of Met suggests an anoretic mechanism and has been associated to decreases in caloric expenditure, reductions of leptin levels and other findings. Despite its beneficial actions, approximately 30% of Met users develop gastrointestinal (GI) adverse effects. The aim of this study was to compare the pharmacokinetic (PK) profiles of Met administered by both the traditional manner (tablets) and by an alternative presentation (Met-Gum), that can be slowly chewed, ingested and that supposedly diminish the GI adverse effects seen with present preparations.
Materials and Methods: We studied 10 healthy volunteers (6 M, 4 F) mean age 30 and 29.8; mean BMI 23.9 and 21.49. Met tablets (Glucophage 850 mg Merck) were purchased; Met-Gum (214.5 mg/unit) were donated by Generex Biotechnology, Canada. Met concentrations were sequentially measured in: **a)** a 12-hour period after administration of an 850 Met tablet; **b)** a 24-hour period after 429 Met-Gum (2 units); and **c)** a 24-hour period after 429 tablet (fraction of the larger tablet) administration. Plasma Met concentrations were determined by HPLC (HPLC time: 6 minutes; retention time of Met and internal standard atenolol: 3.4 and 2.9 minutes respectively. Frozen (−70°) experimental specimens were unthawed for 1 hour before the assay. Removal of proteins and lipids from the biological matrix were achieved prior to HPLC.
Results: Results are shown in the graph.
Conclusion: The Metformin Gum displays a PK profile similar to that of tablets and shows promise as an alternative method for delivery of this biguanide.



Supported by Generex Biotechnology Corp.

0798

Rimonabant improves cardiometabolic risk factors in overweight/obese patients with poorly controlled type 2 diabetes (HbA_{1c}≥8%) on monotherapy with metformin or sulfonylureas

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Background and Aims: Rimonabant, the first selective CB_1 receptor blocker, significantly improved HbA_{1c}, fasting glucose, triglycerides, HDL-cholesterol, body weight and waist circumference in a randomised clinical trial in overweight/obese patients with type 2 diabetes treated with metformin (65%) or sulfonylureas (35%) (RIO-Diabetes [N=1047]). Baseline HbA_{1c} was 7.3% in this study. At 1 year, changes in mean HbA_{1c} were −0.1% and −0.6% with rimonabant 5 and 20 mg/day vs+0.1% for placebo (p=0.034 and p<0.001 vs placebo, respectively). In this analysis, the benefits of rimonabant in the subset of patients with poor glycaemic control (HbA_{1c} ≥8%) were assessed.
Material and Methods: We evaluated the efficacy and tolerability of rimonabant therapy in patients with HbA_{1c} <8% (N=833) or ≥8% (N=188) at baseline. All patients received a hypocaloric diet (600 kcal/day deficit) throughout the study in addition to study treatment with either placebo or rimonabant (5 mg/day or 20 mg/day).
Results: Compared with placebo, rimonabant 20 mg/day significantly decreased body weight and waist circumference from baseline to 1 year in both patient subgroups, as well as improving glycaemic and lipid risk factors (Table). In the HbA_{1c}≥8% group, 30.2% of patients receiving rimonabant 20 mg/day had HbA_{1c} <7% at 1 year compared with 11.1% of those receiving placebo (p=0.012). The corresponding proportions of patients with HbA_{1c} <6.5% were 14.3% and 1.9% (p=0.02). Within the HbA_{1c} <8% and≥8% groups, respectively, 5.4% and 6.6% of patients receiving placebo discontinued therapy as a result of a treatment-emergent adverse events compared with 15.6% and 13.6% of patients receiving rimonabant 20 mg/day. Among the adverse events leading to discontinuation with rimonabant 20 mg/day, those already reported in the previous papers, i.e. psychiatric disorders, nervous system disorders and gastrointestinal disorders, were reported with the following frequency in patients with HbA_{1c} <8% or ≥8%, respectively: 6.3% vs 1.5%; 1.9% vs 4.5% and 3.7% vs 0%.
Conclusion: Rimonabant 20 mg/day improved glycaemic control irrespective of patients' baseline HbA_{1c} levels. In patients with HbA_{1c}≥8% (who were already receiving metformin or a sulfonylurea) reduction from baseline in HbA_{1c} of 1.1% was observed with rimonabant 20 mg/day, along with significant improvements in HDL-cholesterol and triglycerides. Rimonabant 20 mg/day was generally well tolerated. These findings support the use of rimonabant 20 mg/day for improving glycaemic control and reducing cardiometabolic risk in overweight/obese patients with type 2 diabetes.

	HbA_{1c} <8%		HbA_{1c} ≥8%	
	Placebo	Rimonabant 20 mg/day	Placebo	Rimonabant 20 mg/day
Mean change from baseline at 1 year in:	(N=279)	(N=269)	(N=61)	(N=66)
Body weight (kg)	−1.4	−5.5***	−1.6	−4.9***
Waist circumference (cm)	−1.9	−5.5***	−2.0	−4.3*
HbA_{1c} (%)	0.2	−0.5***	−0.3	−1.1***
Fasting glucose (mmol/L)	0.5	−0.4***	−0.3	−1.7*
Fasting insulin (μIU/mL)	0.6	−0.5	0.4	−1.3
Triglycerides (%)	8.9	−6.2***	−0.4	−21.3***
HDL-cholesterol (%)	7.2	15.4***	7.1	16.3*

Data are for ITT population, last observation carried forward.
*p<0.05; ***p≤0.001 vs placebo.

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0799

Rosiglitazone stimulates AMP-activated protein kinase mediated nitric oxide synthesis in cultured human aortic endothelial cells

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Endothelial dysfunction, as assessed by reduced nitric oxide (NO) bioavailability, is associated with Type 2 diabetes and vascular disease. Thiazolidinediones improve insulin resistance and are used in the management of Type 2 diabetes. Incubation of cultured endothelial cells with thiazolidinediones has been reported to stimulate NO production but the precise mechanism remains uncertain. It has been recently demonstrated that AMP-activated protein kinase (AMPK) regulates NO synthesis in cultured endothelial cells via phosphorylation of Ser1177 on eNOS. We have determined the effects of thiazolidinediones on AMPK activity and NO synthesis in cultured human aortic endothelial cells. Incubation of endothelial cells with the thiazolidinedione, rosiglitazone led to a rapid, dose-dependent stimulation of NO synthesis. Moreover, rosiglitazone stimulated AMPK activity, phosphorylation of eNOS at Ser1177 and phosphorylation of acetyl-CoA carboxylase. Rosiglitazone stimulated NO synthesis was inhibited in

cells infected with adenovirus expressing a dominant-negative AMPK mutant. Rosiglitazone-stimulated AMPK activity was not inhibited by the PPARγ inhibitor, GW9662, indicating that the effect of rosiglitazone on AMPK activity was independent of PPARγ. These studies demonstrate that thiazolidinedione drugs stimulate NO synthesis through an AMPK-dependent mechanism in cultured human aortic endothelial cells. Further investigation of the way in which thiazolidinediones affect intracellular signaling in vascular endothelial cells may facilitate more selective treatments for the management of diabetes and vascular disease.

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0800

Tesaglitazar improves glucose and lipid responder rates and goal attainment in patients with type 2 diabetes

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Background and Aims: Tesaglitazar (GALIDA™) is a dual PPAR α/γ agonist in development for the treatment of glucose and lipid abnormalities associated with type 2 diabetes and insulin resistance. GLAD (Glucose and Lipid Assessment in Diabetes) was a randomized, double-blind, placebo-controlled dose-finding study of tesaglitazar in type 2 diabetes which included a pre-planned analysis to determine responder rates and goal attainment for fasting plasma glucose (FPG) and lipid variables.

Materials and Methods: Patients received once-daily tesaglitazar (0.1, 0.5, 1.0, 2.0 or 3.0 mg), placebo or open-label pioglitazone 45 mg (as a therapeutic benchmark) for 12 weeks. Responder rates were defined a-priori as the proportion of patients achieving minimum changes in FPG (−1.7 mmol/L), triglycerides (TG; −30%) and HDL-C (+15%) from baseline to treatment end. The proportion of FPG responders in each tesaglitazar dose and placebo were compared using a 2-sided Fisher's Exact test at the 5% significance level while lipid responders were summarized descriptively. The goal attainment target for FPG was based on the level for diagnosis of type 2 diabetes (FPG <7.0 mmol/L) and lipid goals were TG <1.7 mmol/L and HDL-C>1.0 mmol/L. Goal attainment data were summarized descriptively.

Results: Baseline characteristics for randomized patients (n=500) were similar between treatment groups: FPG 9.5 (SD 1.9) mmol/L; TG 2.1 (1.1) mmol/L; HDL-C 1.1 (0.3) mmol/L; BMI 30.7 (4.7) kg/m^2. Tesaglitazar 1.0 mg resulted in statistically significant reductions in FPG of 2.28 mmol/L, in TG of 32.9% and increases in HDL-C of 15.0%. At baseline 3.7%, 41.0% and 53.8% of patients were already within FPG, TG and HDL-C goals, respectively. Tesaglitazar dose-dependently increased FPG responder rates, which were significantly greater for tesaglitazar≥0.5 mg vs placebo (P<0.0001) (Table). Dose-dependent improvements in responder rates were also shown for TG and HDL-C following treatment with tesaglitazar and these improvements were greater than placebo for tesaglitazar doses of 0.5 mg and above (Table). Greater proportions of tesaglitazar-treated patients attained glucose and lipid treatment goals than placebo recipients (Table). Responder and goal attainment rates were numerically similar for FPG and higher for lipid variables with tesaglitazar 1.0 mg than with pioglitazone 45 mg.

Conclusions: Tesaglitazar treatment produced marked, dose-dependent increases in responder rates and in goal attainment for glucose and lipid measures in diabetic patients. The 0.5- and 1.0-mg doses of tesaglitazar are being studied in Phase III trials in patients with type 2 diabetes.

Variable (pre-specified change/goal)	Placebo	Tesaglitazar (mg)					Pioglitazone 45 mg[†]
		0.1	**0.5**	**1.0**	**2.0**	**3.0**	
n (ITT)	67	71	72	69	69	69	68
Responders							
FPG (≥1.7 mmol/L?)	9	7	38*	45*	65*	81*	50
TG (≥30%?)	10	11	32	51	65	62	21
HDL-C (≥15%?)	22	20	33	64	65	64	38
Goal attainment (in patients not at goal at baseline)							
FPG (<7.0 mmol/L)	3	12	24	40	58	67	28
TG (<1.7 mmol/L)	9	14	42	61	61	77	29
HDL-C (>1.0 mmol/L)	32	35	53	58	63	52	38

*P<0.0001
[†]No statistical comparisons were made between pioglitazone and other groups

Supported by AstraZeneca

0801

Efficacy of vildagliptin combined with pioglitazone in patients with type 2 diabetes

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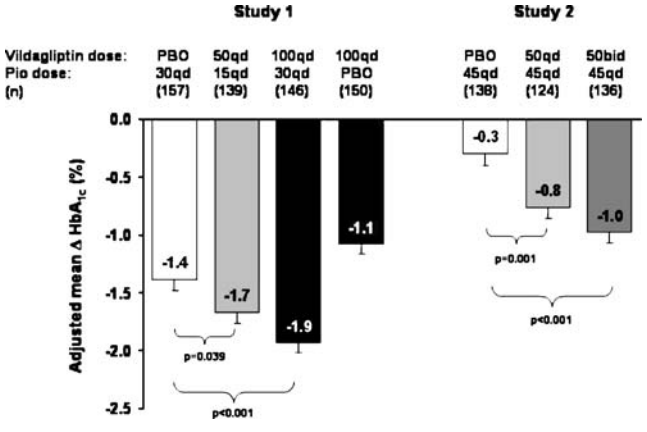
Background and Aims: Vildagliptin is a DPP-4 inhibitor that improves glycemic control by increasing pancreatic α- and β-cell responsiveness to glucose. Vildagliptin is effective and well-tolerated when given as monotherapy or in combination with metformin, but its effects when combined with a thiazolidinedione (TZD) are unknown. The aim of the present study was to assess the efficacy and tolerability of vildagliptin combined with pioglitazone (Pio) in patients with type 2 diabetes (T2DM)
Materials and Methods: Two 24-wk, multicenter, randomized, double-blind, parallel group studies were performed. Study 1 compared vildagliptin 100 mg qd, Pio 30 mg qd, vildagliptin 100 mg qd+Pio 30 mg qd and vildagliptin 50 mg qd+Pio 15 mg qd in drug-naïve patients with T2DM. Study 2 compared vildagliptin 50 mg qd or bid and placebo (PBO) in patients with inadequate glycemic control (HbA_{1c}=7.5–11%) on TZD monotherapy. All patients in Study 2 received Pio 45 mg for≥4 wk prior to randomization and continued Pio 45 mg qd throughout the study.
Results: At baseline (BL), HbA_{1c} averaged 8.6–8.8% in all treatment groups. As shown below, vildagliptin produced a statistically significant and clinically meaningful improvement in glycemic control in combination with Pio, whether combined with lower doses (15 or 30 mg qd) as initial pharmacotherapy as in Study 1, or added to a maximum dose (45 mg qd) as in Study 2.



In drug-naïve patients with BL HbA_{1c}>9.0% (in Study 1), the change from BL (mean=10.0%, n=54) in patients receiving initial combination therapy with vildagliptin 100 mg qd+Pio 30 mg qd averaged −2.8±0.2% (ie, a mean reduction in HbA_{1c} of 2.8%). All treatments were well-tolerated, with a similar number of patients in each treatment group reporting any adverse event (ranging from 46% of those receiving vildagliptin 50 mg qd+Pio 15 mg qd to 56% of those receiving vildagliptin 50 mg qd+Pio 45 mg qd).
Conclusions: Vildagliptin is highly effective and well-tolerated when combined with pioglitazone.
Supported by Novartis

0802

Sustained efficacy and reduced hypoglycaemia with vildagliptin added to insulin in patients with type 2 diabetes

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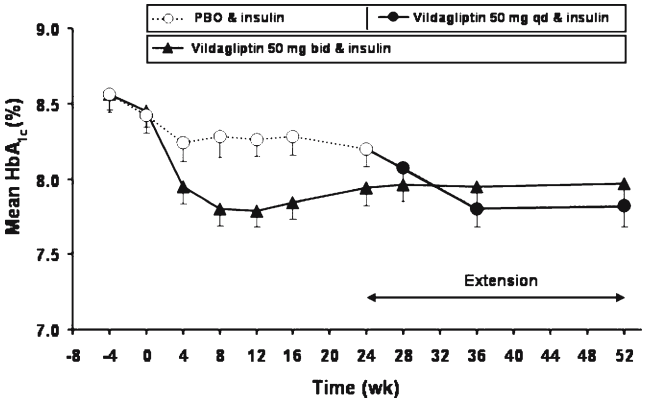
Background and Aims: Vildagliptin is a DPP-4 inhibitor that lowers HbA_{1c} in patients (pts) with type 2 diabetes (T2DM) when given as monotherapy or combined with metformin, but its efficacy in pts with more advanced disease requiring insulin (Ins) is unknown. The aim of this study was to assess the efficacy and tolerability of one-year treatment with vildagliptin added to Ins in pts with T2DM.
Methods: This was a 28-wk extension of a 24-wk, randomized, double-blind, parallel-group study in pts with T2DM inadequately controlled (HbA_{1c} 7.5–11%) by Ins monotherapy (>30 U/d). In the 24-wk core study pts received vildagliptin 50 mg bid (n=125) or placebo (PBO, n=131) added to Ins. In the 28-wk extension, pts who received PBO during the core study switched to vildagliptin 50 mg qd (n=104), while vildagliptin-treated pts from the core study maintained the 50 mg bid regimen (n=96). All pts maintained Ins therapy, but adjustments to dose regimen were allowed.
Results: At baseline (BL) mean age=59 y, BMI=33.0 kg/m^2, disease duration=14.6 y, FPG=9.3 mM and HbA_{1c}=8.5%. Mean duration of Ins use was 76 mo and mean dose was 82 U/d. Eighty-three percent of pts completed the core study and 87.5% of pts in the extension completed 52-wk treatment. In the core study, the adjusted mean change (AMΔ) in HbA_{1c} in vildagliptin-treated pts was −0.5±0.1% and a modest PBO effect was noted (AMΔ HbA_{1c}=−0.2±0.1%), with the between-group difference being significant (P=0.022). Greater efficacy was seen in the>30% of pts age≥65 y for whom the between-group difference (−0.7±0.1%) was highly significant (P<0.001). During the core study, hypoglycemic events were less common and less severe in vildagliptin-treated pts (33 pts, 113 events, 0 severe) than in those receiving PBO (45 pts, 185 events, 6 severe). As depicted below, the efficacy of vildagliptin 50 mg bid added to Ins was maintained throughout the extension and in pts switched from PBO to

vildagliptin 50 mg qd, HbA_{1c} decreased promptly and the improvement was maintained through Wk 52. The mean ΔHbA_{1c} from Wk 24 to endpoint in these pts was −0.4±0.1% (P<0.001 vs Wk 24).



In the 32 pts aged≥65 y in the vildagliptin 50 mg bid arm of the extension, the mean ΔHbA_{1c} from BL to endpoint was −0.9±0.1%. The protective effect of vildagliptin against hypoglycemia was also maintained in the extension. During the whole study, the incidence of hypoglycemia, expressed as events/pt/y was 1.78, 1.80 and 2.66 in the vildagliptin 50 mg qd, 50 mg bid and PBO groups, respectively. The overall incidence of side effects was similar in pts receiving vildagliptin vs PBO.

Conclusion: Adding vildagliptin to Ins in pts with T2DM is well-tolerated and improves glycemic control, with reduced hypoglycemia, particularly in older individuals. Both the greater efficacy in older pts and the protective effect against hypoglycemia are maintained through 52-wk treatment. The mechanism(s) underlying these observations may reflect improved α-cell glucose sensing and merit further study.

Supported by Novartis

0803

Over 75% of patients with type 2 diabetes reached HbA_{1c} <7% by adding biphasic insulin aspart 30 to optimized metformin and pioglitazone treatment

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Physicians often are reluctant to initiate insulin therapy in subjects with type 2 diabetes particularly when oral therapies are not optimized. This randomized, treat-to-target, 34-week, parallel-group study of BIAsp 30 (30% soluble and 70% protaminated insulin aspart) was conducted in insulin-naive, type 2 diabetes patients with HbA_{1c} 7.5–12.0% who were taking any two oral antidiabetic agents. BIAsp30 was compared for efficacy and safety when added to an optimized treatment of metformin and pioglitazone (met+pio) to optimized treatment with met +pio without insulin. During an 8-week run-in, treatment was changed to met and pio, and doses were adjusted to 2500 mg/day and 30 or 45 mg/day, respectively. After randomization, BIAsp 30 was initiated at 6 U BID (prebreakfast and presupper) and titrated to target blood glucose (BG) (4.4–6.1 mmol/L) by an algorithm-directed forced titration. At the end of the study, subjects treated with BIAsp 30+met+pio (n=93) had a mean HbA_{1c} reduction that was significantly greater than the mean reduction for subjects treated with met+pio (n=88) (1.5% vs. 0.2%, p<0.0001 between groups). Subjects treated with BIAsp 30+met+pio were more likely to reach HbA_{1c} targets of≤6.5% and <7.0% than with met+pio only (odds ratios=12.3 and 11.1, respectively). Final HbA_{1c} and fasting plasma glucose (FPG) values were significantly lower for the BIAsp 30 group, as compared to the met+pio group (Table 1). More subjects treated with BIAsp 30 were able to reach the aggressive HbA_{1c} goals of≤6.0% and≤5.5%.

Table 1.

		BIAsp 30+Met+Pio	Met+Pio
HbA_{1c} (mean±SD)	Baseline	8.1±1.0	7.9±0.9
	End of Study (EOS)	6.5±1.0	7.8±1.2
% with HbA_{1c} <7.0%	EOS	76.3	24.1
% with HbA_{1c}≤6.5%	EOS	59.1	11.5
% with HbA_{1c}≤6.0%	EOS	33.3	2.3
% with HbA_{1c}≤5.5%	EOS	14.0	0
FPG, mmol/L	EOS	7.2±2.8	9.0±2.3

The rate of minor hypoglycemia (defined as BG <3.1 mmol/L) in the BIAsp 30 group was significantly greater than in the met+pio group (8.3 vs. 0.1 events/year, p<0.001). Both groups gained weight during treatment (BIAsp 30, 4.6±4.3 kg; met+pio, 0.8±3.2 kg, p<0.05). Peripheral edema was reported by 10% and 12% of subjects in the BIAsp 30 and met+pio groups respectively.

In conclusion, more patients with type 2 diabetes, poorly controlled by 2 oral agents, could achieve currently recommended ADA and IDF/ACE HbA_{1c} targets using BIAsp 30+met+pio than using met+pio alone.

0804

Insulin (INS) add-on to rosiglitazone/metformin (RSG/MET) fixed-dose combination therapy (FDCT) vs. INS switch from RSG/MET FDCT: satisfaction with treatment

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Background and Aims: Greater treatment satisfaction may predict improved medication adherence and long-term glycemic control in type 2 diabetes (T2D). This 24-week, randomized, double-blind study assessed treatment satisfaction in patients with T2D insufficiently controlled on a maximum daily dose of RSG/MET FDCT (8 mg/2000 mg). Following 8-week max dose RSG/MET FDCT run-in, patients received INS supplemental to RSG/MET (INS add-on to RSG/MET) (n=163) or were switched to an INS-only regimen (INS switch) (n=161).
Materials and Methods: The Diabetes Treatment Satisfaction Questionnaire (DTSQ) assessed patient satisfaction with treatment (range 0–36), perceived frequency of hyperglycemia (range 0–6) and perceived frequency of hypoglycemia (range 0–6) in INS add-on to RSG/MET vs. INS switch groups.
Results: INS add-on to RSG/MET FDCT was superior to INS switch in reducing HbA1c (mean adjusted treatment difference −0.65%, $p<0.0001$) while requiring less INS. At study end, mean treatment differences for change from screening and baseline in treatment satisfaction were significant (1.4 and 1.5 points, respectively, $p<0.05$) and favored INS add-on to RSG/MET. Perceived frequency of hyperglycemia decreased (improved) during the study in two groups. Mean treatment differences for change from screening and baseline in perceived frequency of hyperglycemia were significant (0.7 points, $p<0.0006$ and 0.8 points, $p<0.0001$, respectively) showing greater improvements in INS add-on to RSG/MET. There were no between-group differences in perceived frequency of hypoglycemia.
Conclusion: In patients with T2D, significant positive changes in treatment satisfaction and perceived frequency of hyperglycemia along with stable perceived frequency in hypoglycemia scores add value to superior glycemic control afforded by lower doses of INS added to RSG/MET FDCT compared to switch to higher doses of INS alone. Increased satisfaction with treatment could benefit long-term medication adherence and sustainable glycemic control.
Supported by GSK

PS 63

Thiazolidinediones in combination therapy

0805

Long-term glycaemic effects of pioglitazone in triple oral therapy: Results from PROactive

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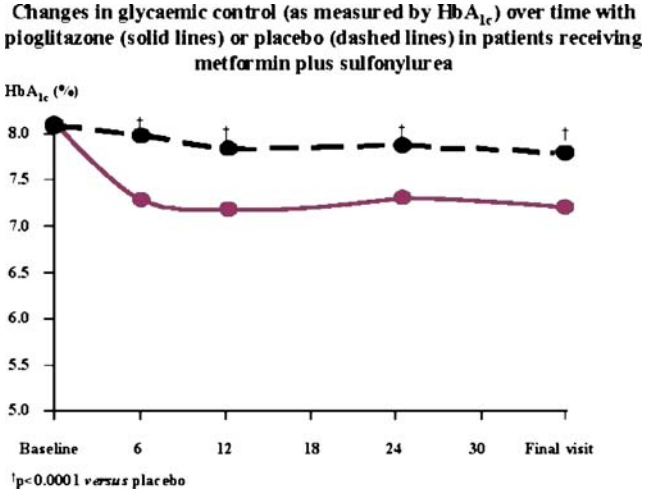
Background and Aims: Type 2 diabetes is a progressive disease and its treatment eventually requires multiple-agent therapy, including insulin. PROactive was a cardiovascular outcome study, which examined the effects of pioglitazone on patients whose type 2 diabetes had been diagnosed on average 9.5 years before study entry. Little data exists on the benefits of using triple oral therapy (metformin +sulfonylurea+thiazolidinedione) in those patients failing dual oral treatment. This subanalysis evaluates the long-term glycaemic effects of pioglitazone add-on therapy in patients with type 2 diabetes and macrovascular disease who entered on metformin plus sulfonylurea.
Materials and Methods: PROactive randomised patients to either pioglitazone or placebo, in addition to other glucose-lowering and cardiovascular medication, which was adjusted as necessary to treat to IDF target. Pioglitazone doses were force-titrated from 15 mg to 45 mg. Mean follow-up was 34.5 months. In total, 1314 patients entered the study on metformin plus sulfonylurea. Within this cohort, mean baseline HbA_{1c} values were similar between groups (pioglitazone: 8.16%; placebo: 8.14%).
Results: Significantly greater reductions in HbA_{1c} were seen with pioglitazone (−0.9%) compared with placebo (−0.4%, P<0.0001). The significant improvement in HbA_{1c} with pioglitazone *versus* placebo was shown with the following changes in associated glucose-lowering medication: more pioglitazone patients had either metformin or sulfonylurea dropped from their regimen (16%) and fewer had insulin added to their regimen (16%) than did placebo patients (8% and 31%, respectively). The metformin dose increased by 19 mg with pioglitazone *versus* 228 mg with placebo (P<0.0001). Sulfonylurea doses decreased or were unchanged in the pioglitazone group (−1.4 mg for glibenclamide *versus* −0.2 mg for placebo, P=0.013; −33 mg for gliclazide *versus* −23 mg for placebo, P=0.270; 0 mg for glimepiride *versus*+0.6 mg for placebo, P=0.009). Oedema occurred in 29% of patients in the pioglitazone group *versus* 17% in the placebo group (P<0.0001) and hypoglycaemia occurred in 27% in the pioglitazone group

versus 20% in the placebo group (P=0.0013). There was a weight increase of 4.1 kg in the pioglitazone group and a decrease of 0.7 kg in the placebo group (P<0.0001).



Conclusion: Little data exist on the benefits of triple oral therapy in patients with type 2 diabetes. Adding pioglitazone to a dual oral therapy regimen (metformin+sulfonylurea), thus resulting in a triple oral regimen, resulted in a sustained improvement in glycaemic control and a reduced need for insulin, with a good overall safety profile.

0806

Pioglitazone reduces insulin requirements and improves glycaemic control in insulin-treated patients with type 2 diabetes: results from PROactive

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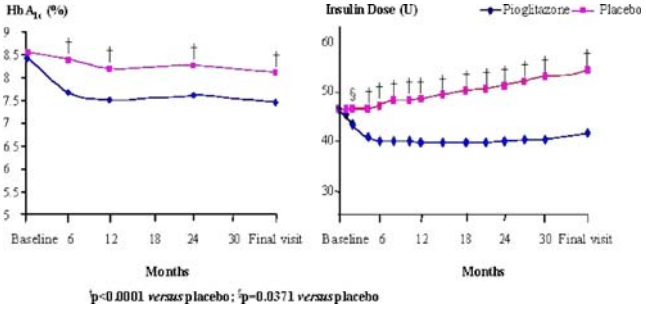
Background and Aims: Type 2 diabetes is a progressive disease with associated insulin resistance and diminished insulin production. Treatment typically requires multiple agents, including insulin. PROactive was a study designed to evaluate the effects of pioglitazone on cardiovascular outcomes in 5,238 high-risk patients with type 2 diabetes. We examined insulin requirements and glycaemic control in the one-third of patients (n=864 in the pioglitazone group; n=896 in the placebo group) who were treated with insulin at baseline in PROactive.

Materials and Methods: PROactive was a randomised, double-blind, placebo-controlled outcome study in patients with type 2 diabetes and macrovascular disease randomised to pioglitazone or matching placebo, in addition to their existing glucose-lowering and cardiovascular medication. Doses of pioglitazone were force-titrated to a dose of 45 mg, if tolerated. Mean follow-up was 34.5 months. Within the cohort of patients receiving insulin at baseline, baseline mean daily insulin doses and HbA_{1c} values were similar between treatment groups (pioglitazone: 47 U/d and 8.4%; placebo: 47 U/d and 8.5%). In both groups at baseline, insulin was combined with oral monotherapy with metformin in 53% and sulfonylurea in 24% of patients and with dual therapy with metformin+sulfonylurea in 12%.

Results: There was a rapid and sustained decrease in insulin dose with pioglitazone, whereas there was a progressive increase with placebo. By study end, the mean insulin dose was lower with pioglitazone (42 U/d) than with placebo (55 U/d; P<0.0001); nevertheless, a greater decrease in HbA_{1c} was observed with pioglitazone (−0.93%) compared with placebo (−0.45%; P<0.0001). At final visit, insulin had been discontinued in 9% of patients in the pioglitazone group *versus* 2% in the placebo group (P<0.0001). The distribution of patients on oral/insulin combined therapy remained similar in both groups: metformin alone in 47% *versus* 52%, sulfonylurea alone in 16% *versus* 16%, and metformin+sulfonylurea in 10% *versus* 11%, in the pioglitazone group *versus* the placebo group, respectively (P=NS). There were differences in oedema (pioglitazone: 31%; placebo: 18%; P<0.0001) and hypoglycaemia (pioglitazone: 41%; placebo: 29%; P<0.0001), but there were no other differences in the safety profiles between the pioglitazone and placebo groups (with or without other treatments).



Conclusion: Pioglitazone reduced the number of patients on insulin and the mean daily insulin dose while providing better glycaemic control than placebo.

0807

Glycaemic control in patients with type 2 diabetes: the effects of pioglitazone vs glyburide in a 3-year randomized double-blind trial

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Background and Aims: Little long-term data comparing the effects of a sulfonylurea to a thiazolidinedione on glycemic control have been published. UKPDS has shown that because of the progressive nature of type 2 diabetes (T2D), many patients fail initial monotherapy for glycemic control after 3 to 4 years as noted by the terms "sulfonylurea burnout" and "metformin failure." We examined the long-term glycemic benefits of pioglitazone (PIO) compared to glyburide (GLY) in one of the longest studies evaluating the effects of PIO.
Materials and Methods: In this US multicenter, double-blind, randomized comparator study, 2120 patients (18–80 yrs) with T2D were enrolled. Patients on metformin at study entry were allowed to continue this therapy (up to 850 mg bid); all sulfonylurea therapy was discontinued. During the 36-month treatment period, PIO was titrated to 45 mg (in 15 mg intervals) and GLY was titrated to 15 mg (in 5 mg intervals). At the investigator's discretion, metformin was either maximized or added to achieve A1C <7.5%. If A1C goals were not achieved after 3 months, insulin therapy was added.
Results:

Least Squares Mean Change From Baseline in A1C

	n	PIO N=1051 LS Mean % (SD)	n	GLY N=1046 LS Mean % (SD)	Between-groups P-Value
Baseline	782	9.41 (2.07)	850	9.34 (2.01)	0.492
Week 16	683	−0.29 (2.27)	739	−1.12 (1.98)	<0.0001
Week 32	574	−1.11 (2.24)	601	−1.21 (2.07)	0.3522
Week 48	512	−1.59 (2.11)	559	−1.54 (2.11)	0.6167
Week 72	447	−2.07 (2.07)	495	−1.67 (2.10)	0.0002
Week 96	403	−2.17 (2.05)	450	−1.83 (2.07)	0.0008
Week 120	360	−2.08 (1.87)	396	−1.65 (2.13)	<0.0001
Week 144	335	−2.39 (2.11)	355	−1.91 (2.22)	<0.0001
Week 156	323	−2.34 (2.08)	333	−1.85 (2.07)	<0.0001

Conclusion: Treatment groups had similar mean baseline A1C levels (PIO=9.41%, GLY=9.34%). PIO treatment resulted in significantly greater reductions in A1C compared to GLY starting at Week 72 and continued through 3 years of treatment. In conclusion, subjects treated with PIO showed sustained and greater long-term glycemic control compared to GLY.

0808

Evaluation of changes of glycaemic control and lipid parameters in patients with T2DM after one year of treatment with pioglitazone in combination with metformin or sulfonylurea in a Greek observational study

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Background and Aims: Type 2 diabetes mellitus (T2DM) is associated with hypertriglyceridemia, low HDL-c and an increase of small dense LDL-c. This feature of diabetic dyslipidemia is the most well characterized risk factor for increasing atherosclerosis in patients(pts) with T2DM. Evaluate the changes of the glycemic control and of the lipid profile in pts with T2DM treated with Pioglitazone (Pio) in combination with Metformin (Met) or Sulfonylurea (Su).
Materials and Methods: The Clinical Evaluation of the Lipid profile in T2DM treated with oral Agents in combination (ECLA) is a one-year, multicenter Phase IV, open label, prospective study in 2388 T2DM pts poorly controlled with monotherapy and according to the physician's judgement required combined oral treatment. They were assigned in 3 treatment groups: (1) Pio+Su (2) Pio +Met and (3) Su+Met. We report here the data analysis performed after 12 months of follow-up in 2116 (88.6%) pts who completed the study without changing their treatment[Pio+Su(791), Pio+Met(705), Su+Met(620)]. 77.3% from Pio+Su, 72.1% from Pio+Met and 72% from Su+Met were not receiving hypolipidemic treatment.
Results: Fasting plasma glucose (mg/dl) decreased significantly from baseline with Pio+Su (198 to 134) and Pio +Met (194 to 131) compared to Su+Met (189 to 138), representing a decrease of 31.1, 32 and 26.3% respectively ($p<0.0001$ comparing Pio groups to Su+Met). HbA1c decreased significantly in all groups($p<0.0001$) but more in the Pio groups (8.23 and 8.1% becoming 6.65 and 6.61%), compared to Su+Met (7.96 to 6.93%) ($p<0.0001$). Total cholesterol (TC) (mg/dl) baseline mean values were similar in all cohorts (237). After 12 months TC was decreased significantly in all groups ($p<0.0001$), but significantly more in the Pio groups (207) representing a reduction of 12.8% vs 9.9% in the Su+Met (212) ($p<0.0001$). The HDL-c (mg/dl) mean values were 43.8 for Pio+Su, 43.1 for Pio+Met and 43.5 for Su+Met at

baseline. After 12 months an increase was observed in all groups ($p<0.0001$) but significantly more in the Pio groups 8.3–9.5% vs 4.6% in Su+Met ($p=.0003$, $p<0.0001$ correspondingly). Triglycerides (mg/dl) decreased in all groups ($p<0.0001$) but significantly more for the Pio+Su and Pio +Met groups (177 to 141 and 184 to 142) representing a reduction of 20.7 and 21.6% vs 15.1% in the Su+Met (178 to150) ($p<0.0001$). A significant reduction of the LDL (mg/dl) from baseline to 12 months was detected with Pio+Su (157 to 133) and Pio+Met (153 to 132) compared to Su +Met (154 to 137), representing a decrease of 15.2, 14.6 and 11.3% respectively ($p=.0001$ and $p=.005$ comparing Pio+Su and Pio+Met to Su+Met). The atherogenic index i. e. log [TG/HDL-c] decline after 12 months in the Pio+Su and Pio+Met groups (22.6 and 23.3%) vs 15% in Su+Met ($p<0.0001$). The differences observed in the lipid profile, were independent of concomitant administration of hypolipidemic medication and were also documented in the analysis of the 6 months results.

Conclusion: Combined treatment with Pio+Su and Pio +Met over one year, compared with Su+Met in a daily clinical practice setting, resulted in significantly greater improvement in glycemic control and led to a less atherogenic lipid profile, confirming the value of Pioglitazone for the combined treatment of T2DM.

Supported by Pharmaserv-Lilly SACI

0809

Dose dependent improvement of β cell dysfunction by addition of pioglitazone to existing oral treatments under daily routine conditions

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Background and Aims: The degree of β-cell dysfunction in orally treated patients with type 2 diabetes has been shown to correlate with cardiovascular risk and prevalence of macrovascular disease. The ratio of correctly processed secretion product (C-peptide) and the uncleaved intact proinsulin is a good marker for cell function. This study was performed to investigate the effect of addition of different doses of pioglitazone to the existing therapy on β-cell dysfunction under daily life conditions.

Materials and Methods: In total 402 patients were included (223 women, 179 men, age(mean±SD): 61± 11 years, disease duration:6±5 years, BMI: 31.4±5.5 kg/m^2, HbA1c: 7.6±1.1%). Decision about the dose was left to the discretion of the treating physician. Before and after 5 months of treatment, β-cell function was assessed by measurement of fasting C-peptide (CP) and intact proinsulin (iPi) and formation of the fasting CP/iPi ratio. Other observation parameters included HbA1c, fasting glucose, and blood pressure.

Results: Patients who received only 15 mg of Pio (n=32) had a better fasting CP/PI ratio and a lower blood pressure at baseline compared to those receiving 30 mg (n=227) or 45 mg (n=93). While no statistically significant difference between the groups was observed regarding reduction of HbA1c (mean change: −0.7±1.1%, $p<0.001$ vs. baseline) and fasting glucose (−31±43 mg, $p<0.001$), dose dependent improvements could be observed for β-cell dysfunction and the blood pressure assessments as shown in the table.

Conclusion: Addition of pioglitazone to existing oral antidiabetic treatments under daily routine conditions, leads to improvement of glucose control and additional dose dependent improvements of β-cell dysfunction and blood pressure. Dose dependent improvement by pioglitazone in β-cell function (CP/iPi) and blood pressure (BP)

Parameter	15 mg		30 mg		45 mg	
a: $p<0.05$ b: $p<0.001$	baseline	endpoint	baseline	endpoint	baseline	endpoint
CP/iPi ratio	118±105	112±54	107±73	123±82[b]	106±70	131±84[b]
BP syst [mmHg]	136±17	136±17	140±15	135±13[b]	142±17	134±14[b]
BP diast [mmHg]	78±8	79±7	82±9	81±8[a]	84±10	81±7[b]

Supported by Takeda Pharma

0810

Effects of the addition of pioglitazone on insulin resistance, beta cell function and proinsulin/insulin ratio in patients with type 2 diabetes inadequately controlled on metformin and a sulphonylurea

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Background and Aims: Pioglitazone belongs to the class of thiazolidinediones that reduces insulin resistance in the liver, adipose tissue and peripheral muscle. In addition, pioglitazone (PIO) has shown improvements in markers of beta-cell function. We evaluated the effect on endogenous insulin production and markers of beta-cell function of

adding PIO to metformin/sulphonylurea (MET+SU) dual therapy in inadequately controlled, insulin-naïve patients with Type 2 diabetes.

Materials and Methods: This prospective double-blind, placebo-controlled study randomised 299 patients with Type 2 diabetes previously treated with MET (≥1700 mg/day) and SU (maximal dose) for more than 3 months and HbA_{1c} between 7.0% and 9.5%. After a 4-week run-in, patients received either PIO 30 mg once daily or placebo. After 3 months, if HbA_{1c}>6.5%, PIO or placebo was uptitrated to 45 mg for the remaining 4 months. Endogenous insulin production was assessed by measuring fasting plasma insulin (I), intact proinsulin (PI) and C-peptide at baseline and month 7. Insulin resistance and beta-cell function were evaluated using the homeostasis model assessment for insulin resistance (HOMA-IR) and beta-cell function (HOMA-B). The analyses were conducted on data from patients who had received at least 3 months' study treatment.

Results: Overall, 289 patients (mean age 60±9 years, mean duration Type 2 diabetes 12±8 years) were included in the full analysis population.

Conclusion: The addition of PIO to MET+SU dual therapy significantly improved glycaemic control, markers of insulin resistance and beta-cell function in patients with Type 2 diabetes inadequately controlled on dual therapy. Moreover, this double-blind, randomised controlled study has shown for the first time that the addition of PIO to MET+SU decreases PI and improves PI/I ratio, representing a potential additional benefit by reducing this independent cardiovascular risk factor.

Adjusted mean changes±S.E. from baseline at 7 months

	Pioglitazone	Placebo	Treatment difference
Insulin, mmol/L	−14.49±2.57 n=127	−6.67±2.54 n=130	−7.82±3.62; p=0.032
Proinsulin, pmol/L	−4.44±2.74 n=133	11.29±2.68 n=139	−15.75±3.84; p<0.001
Proinsulin/insulin ratio	0.63±0.05 n=126	0.77±0.05 n=128	−0.14±0.07; p<0.04
C-peptide, μg/L	−0.20±0.08 n=129	0.13±0.07 n=137	−0.33±0.11; p=0.002
HOMA-IR	−2.01±0.19 n=115	−0.43±0.18 n=119	−1.58±0.26; p<0.001
HOMA-B	24.24±7.03 n=114	−2.26±6.88 n=119	26.50±9.84; p=0.008

Supported by Laboratoires Takeda, France

0811

Fixed-dose combination of rosiglitazone and glimepiride provides superior glycaemic control compared with glimepiride or rosiglitazone monotherapy in drug-naïve individuals with type 2 diabetes mellitus

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Background and Aims: Conventional management of type 2 diabetes (T2DM) continues to involve maximizing the use of a monotherapy before introducing a second agent, despite the high incidence of therapeutic failure with this strategy. Recent evidence suggests that the earlier use of combination therapy may improve long-term glycemic control. The aim of this study was to investigate whether combination therapy with rosiglitazone (RSG) and glimepiride (GLIM) was more effective than either agent alone in drug-naïve individuals with T2DM.

Materials and Methods: In this multi-centre study, drug-naïve participants with T2DM were randomized to receive 28 weeks of double-blind treatment with GLIM [1–4 mg once daily (OD)], RSG (4–8 mg OD), RSG/GLIM fixed-dose combination (FDC) A (4 mg/1–4 mg OD) or RSG/GLIM FDC B (4–8 mg/1–4 mg OD). Treatment was started on the lowest dose and uptitrated at 4-week intervals up to 12 weeks unless mean daily glucose level of <6.1 mmol/l. Efficacy was assessed by determining HbA_{1c} levels at the end of the study.

Results: A total of 901 patients were involved in the study, and 883 patients constituted the efficacy population. Baseline characteristics of mean age (53.96±10.99 years), BMI (31.68±6.41 kg/m^2), HbA_{1c} (9.07±1.31%) and duration of T2DM (2.89±3.87 years) were comparable between treatment groups. Both FDC regimens gave superior glycemic control compared with RSG or GLIM alone. A statistically greater proportion of participants achieved the ADA target of HbA_{1c} <7% with FDC A (75%) or FDC B (72%) compared with GLIM (49%) or RSG (46%) monotherapy. Furthermore, the more aggressive target of <6.5% was also achieved by more patients with FDC A (56%) or FDC B (54%) compared with GLIM (32%) or RSG (30%) alone. Rapid FPG reductions were observed in both FDC regimens, with a statistically greater reduction compared with either GLIM or RSG monotherapy. Both FDCs were generally safe and well tolerated, with adverse event profiles as expected from their component therapies. Despite improved glycemic control, there was no increase in hypoglycemic events compared with GLIM alone (FDC A 3.6%, FDC B 5.5%, GLIM 4.1%).

	GLIM n=221	RSG n=227	FDC A n=221	FDC B n=214
HbA_{1c} %				
Baseline	9.0±1.3	9.1±1.3	9.0±1.3	9.2±1.3
Mean Δ from baseline	−1.7±1.4	−1.8±1.5	−2.4±1.4*†	−2.5±1.4*†
FPG mmol/l				
Baseline	11.7±3.9	11.8±3.7	11.5±3.2	11.9±3.4
Mean Δ from baseline	−2.3±3.7	−3.2±3.2	−3.9±3.2*†	−4.4±3.2*†

*p<0.0001 vs. GLIM; †p<0.0001 vs. RSG

Conclusion: This study shows that the use of FDC (RSG/GLIM) is an effective and well tolerated initial therapy for drug-naïve T2DM individuals. These data also demonstrate the superior efficacy of FDC (RSG/GLIM) compared with RSG or GLIM monotherapy in this patient population, with more individuals achieving glycemic targets.

0812

Glycaemic response of pioglitazone in dual combination and triple therapy in patients with type 2 diabetes mellitus

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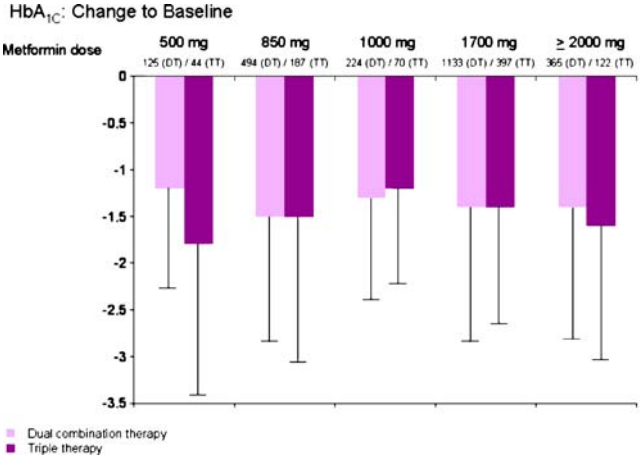
Background and Aims: Type-2-Diabetes mellitus (T2DM) is a disease with progressively deteriorating glycaemic control. Insulin resistance (IR) and interrelated declining betacell function (BCF) are believed to be the core defects of the disease. Pioglitazone (PIO) is a PPARγ agonist offering unique and sustained glucose-lowering benefits via ameliorating IR and BCF. Clinical studies revealed that combination therapy of PIO with Metformin (MET) could be the preferred treatment option of choice offering additional metabolic control combined with a favourable safety profile. Current evidence suggests that early and aggressive combination therapy will slow the progression of the disease and its complications. There are limited data available for the effects of PIO in triple therapy. In addition data are lacking whether the glycaemic response is related to the dose of the concomitant antihyperglycaemic therapy at baseline. Therefore we studied the glucose lowering effect of PIO in a "real world situation" in a cohort of rather unselected patients with T2DM.
Materials and methods: open-label, non-controlled observational study performed according to the guidelines of the German Federal Health Ministry. Combination therapy with PIO 30 mg qd was initiated for a mean observation time of 17±5 weeks. PIO therapy was initiated in 2,341 patients as add-on to MET and in 820 patients as third agent. PIO and MET dosage were aimed to keep stable throughout the observation period. Primary parameters were for different BL levels adjusted changes of HbA1C and fasting Glucose levels (FG) compared to that at BL. All results are expressed as means±SE. Wilcoxon-test was used to test statistical significant difference for HbA1c and blood sugar levels. Kruskal-Wallis-test was used to test intergroup differences concerning the used dosages of concomitant antidiabetic medication.
Results: HbA1C declined from 8.4%±1.4 to 7.0%±1.0 (p<0,001) and 8.8%±1.5 to 7.3%±1.1 (p<0,001) in dual combination therapy (DT) and triple therapy (TT), respectively. Compared to BL FG levels could be reduced by 50.8 mg/dl (DT) and 53.5 mg/dl (TT).
Overall 51.9% (DT) and 40.4% (TT) reached a HbA1C goal of <7%. Only 1 (0.03%) reported hypoglycaemia. A mean weight gain of 1.1 kg was observed.
Conclusion: PIO revealed significant benefits for glycaemic control in DT as well as TT. These benefits could be achieved independent of MET doses at BL. The combination approach offers also a favourable safety profile which could be relevant for patient acceptance in the long-term.



Supported by Takeda Pharma

0813

The efficacy and safety of pioglitazone in addition to SU or metformin vs fixed combination of metformin plus glibenclamide on diabetic dyslipidaemia

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Background and Aims: The effects of co-administration of pioglitazone with either metformin or sulphonylurea on diabetic dyslipidemia have been described in recent publications. Little or no data exists on the comparative effects between a fixed combination of metformin +sulphonylurea and the co-administration of pioglitazone and metformin or sulphonylurea. This study aims to present data on the comparative effectiveness of these different treatment regimes on diabetic dyslipidemia.
Materials and Methods: This was a prospective open, three arm comparative randomised study in patients with Type 2 diabetes previously treated with maximal permitted/ tolerated does of metformin or sulphonylurea for at least 3 months and HbA1c between 7.5% and 11%. After a 1-week run-in period, patients received either pioglitazone 15 mg once daily as add-on in patients receiving metformin or sulphonylurea or a fixed dose combination of metformin 400 mg and glibenclamide 2.5 mg twice a day. Pioglitazone could be increased to 30 mg/day and the fixed combination could be increased to 3 times a day at 15 days, 2 or 4 months if the plasma glucose was>140 mg/dl or HbA1c was>7.5%. HDL-cholesterol, total cholesterol and triglycerides were measured at baseline and 6 months.
Results: 398 patients were screened and 250 were randomised into the trial. 103 patients were put Ùnto the pioglitazone+metformin group, 77 into the pioglitazone +sulponylurea group and 80 into the fixed dose metformin +glibenclamide group. HDL-cholesterol was significantly raised by 2.3 mg/dl from baseline in the pio+met group whereas there was a significant decrease of −3.3 mg/dl in the fixed dose combination group at 6 months. There was a significant difference in favour of both pio+met and pio +SU vs fixed dose regarding HDL change. There were no differences between the groups for total cholesterol. A significant drop in triglycerides was only seen in the pio +SU group (28.3 mg/dl).
Conclusion: This study showed that using pioglitazone in co-administration with either metformin or sulphonylurea effectively improved diabetic dyslipidemia as compared with a worsening of HDL and no effect on other lipid parameters with the fixed dose combination. All three treatments were well tolerated with an increase in body weight seen in all three groups.
Supported by Takeda Italy

PS 64

Diabetes in the “real world”

0814

Frequency and motives of blood glucose self-monitoring in type 1 diabetes: association with glycaemic control, complications, and patient characteristics

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Background and Aims: Recommendations for self-monitoring of blood glucose (SMBG) from the DCCT have not been implemented with the same intensity as recommendations for insulin therapy. We assessed frequency and motives of SMBG and the relationship between SMBG and clinical, behavioural and demographic characteristics.
Material and Methods: Cross-sectional Danish-British multicentre survey of 1076 consecutive patients with type 1 diabetes who completed a detailed questionnaire on SMBG and related issues. The key variables were test frequency and motive.
Results: SMBG was performed daily by 39% of the patients, in series one or more days per week by 20%, as single tests more than once a week by 17% and less than weekly by 24%. The incentives to test were suspicion of hypo- or hyperglycaemia (33%), routine checks (44%), or a combination (23%). The following parameters remained significant in a stepwise multivariate analysis and were related with daily SMBG: higher age, female gender, high educational level, non-smoking, 4-dose insulin regimen, low HbA1c, more feelings in the preceding week, unawareness, autonomic neuropathy, and long duration of diabetes. Routine testing was associated with higher age, longer duration of diabetes, and lower HbA1c in the multivariate analysis.
Conclusion: Patient compliance regarding SMBG is suboptimal and too many patients do not perform SMBG routinely. Patient groups needing more attention are young men with a short duration of diabetes, who have a lower educational level, do not think much of their diabetes, and are smokers.

0815

Self-monitoring of blood glucose in type 2 diabetic patients not on insulin treatment: waist of money or tool to reach the glycaemic targets?

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Background and Aims: There is no agreement whether self-monitoring of blood glucose (SMBG) is a tool to improve clinical outcomes in type 2 diabetic patients not on insulin. For this reason, limitations in BG strips reimbursement are applied: in our Region, 4 strips/month are routinely admitted. In this study, we aimed at investigating whether frequency and completeness of SMBG play a role in the attainment of the glycemic targets.
Materials and Methods: We enrolled 273 consecutive type 2 diabetic patients not on insulin, with HbA1c stable in the last 6 months, already on SMBG, and randomized them into 3 groups: Group A (n=96) was invited to continue the usual SMBG by performing one BG profile a month with fasting, 2 h after breakfast, 2 h after lunch and 2 h after dinner BG measurement; Group B1 (n=89) was invited to perform one BG profile every 15 days with fasting, 2 h after breakfast, before lunch, 2 h after lunch, before dinner, 2 hours after dinner BG measurement; Group B2 (n=88) was invited to perform BG profiles as group B1, with the recommendation to call the Diabetes Clinic when BG targets were not reached. The three groups did not differ for gender, age, known diabetes duration, BMI, waist circumference, type of therapy and HbA1c. Patients were followed-up by the same team at 3 month intervals and received the same education and treatment protocol. At 3 and 6 months, SMBG profiles were evaluated and HBA1c was measured.
Results: SMBG was carried out as recommended by 73% of Group A patients and 44% of Groups B1 and B2 patients, which were finally considered together (Group B) since a very few patients of Group B2 called the Diabetic Clinic. Compliant and not compliant patients did not differ for the parameters mentioned in Materials and Methods. Thus, BG and HbA1c were evaluated in the compliant patients of Group A (n=70/96) and B (n=78/177), which presented no significant differences in gender (M/F 41/29 vs 47/31), age (63.1±9.44 vs 65.8±9.2 years), known diabetes duration (10.6±8.9 vs 10.6±8.5 years), BMI (29.1±4.9 vs 28.1±3.5), waist circumference (103.4±12.1 vs 102.1±8.8 cm), HbA1c (7.97±0.72 vs 8.1±0.8%). At 6 months, HbA1c was 7.78±1.05 vs 7.97±0.72% (ns) in Group A and 7.6±0.73 vs 8.08±0.84% (p=0.001) in Group B; no significant change in BG was observed in Group A, whereas in Group B the BG values at 6 months vs baseline were: i)fasting 129.61±27.33 vs 137.5±28.29 mg/dl, p=0.013; ii)2 hours after breakfast 147.62±29.4 vs 158.3±35.86 mg/dl, p=0.004; iii)before lunch, 124.56±25.71 vs 134.99±34.84 mg/dl, p=0.003; iv)2 hours after lunch 157.32± 27.93 vs 163.9±34.38 mg/dl, ns; v) before dinner, 120.27±27.06 vs 129.42±32.8 mg/dl, p=0.037; vi) 2 hours after dinner 153.22±29.42 vs 162.88± 30.08 mg/dl, p=0.002. Therapeutical changes prescribed at the 3 month visit were more frequent in Group B (44.9%) vs Group A (32.8%).
Conclusion: SMBG is a tool to improve BG control in type 2 diabetic patients not on insulin only when performed with an appropriate frequency/completeness, because only in this case it has a sufficient educational value and reduces the therapeutical "inertia". Only 44% of patients, however, are able to do it carefully. Furthermore, this study shows that type 2 diabetic patients not on insulin seldom utilize the availability of the diabetes team to be contacted out of the scheduled visits.
Supported by Piedmont Region

0816

Self monitoring of blood glucose is common in German people with non-insulin treated type 2 diabetes and associated with better long term outcome

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Background and Aims: Randomised controlled trials of self-monitoring of blood glucose (SMBG) for long term outcome in non-insulin treated type 2 diabetes are not feasible. Therefore information from epidemiological cohort studies should be considered.
Materials and Methods: In the German multicentre, retrospective cohort study ROSSO people with type 2 diabetes were followed from diagnosis between 1995 and 1999 until the end of 2003. In order to minimise any bias in patient selection, all diabetic patients of 192 randomly picked practices involved in primary care were screened and included in the analysis if≥45 years of age at diagnosis and treated for at least one year. Patients who received oral antidiabetic drugs (OAD) for at least one year, but no insulin during the observation period or until a clinical endpoint were analysed.
Results: During a mean follow-up of 6.5 years 742 of a total of 1912 patients in the OAD cohort (38.8%) began with SMBG. Initiation of SMBG was preceded by worsening of

metabolic control. People performing SMBG were younger by a mean of 3 years and more often male (56% versus 47% in the no SMBG cohort), but did not differ for most other characteristics including smoking. Persons in the SMBG cohort changed OAD therapy more often (66.3% of patients versus 48.3%, p<0.001). SMBG was not accompanied by more medication for treating hypertension, hypercholesterinaemia or thrombocyte aggregation risk. The composite clinical endpoint comprised diabetes-related morbidity (myocardial infarction, stroke, foot amputation, blindness, or haemodialysis) and all-cause mortality. In the SMBG cohort, significantly fewer persons had an endpoint (9.2%) compared to the cohort without SMBG (13.2%, p=0.038 after multivariate adjustments for baseline differences, confirmed by Kaplan-Meier survival analysis, p=0.002). Stratification of patients by metabolic control, blood lipids or BMI did not reveal a subgroup in which the use of SMBG was not associated with better outcome.

Conclusion: In this retrospective study of patients treated in German primary care centres, more than one third of non-insulin treated type 2 diabetes patients began with SMBG during the first 6 years after diagnosis, apparently in response to deteriorating metabolic control. The use of SMBG was associated with better long-term outcome.

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0817

ASIAD PROJECT: *A*lphabet *S*trategy *I*ndian *A*pplication for *D*iabetes: Effective application of European strategy in outpatient setting in Asia

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Background and Aims: The Alphabet Strategy is an evidence based template that focuses around seven essential bits of effective diabetes care: ***A***dvice, ***B***lood pressure control, ***C***holesterol control, ***D***iabetes control, ***E***ye care, ***F***oot care, ***G***uardian drugs eg. Aspirin, ACE-inhibitors. Onset of diabetes in Asians occurs atleast a decade earlier, thereby increasing the risk of complications. An effective, target driven diabetes care strategy is required to combat the economic burden.

The aim of our study was

1. To assess the application of the Alphabet Strategy of diabetes care to improve health care delivery in outpatient setting in India.
2. To compare the health care delivery standards of care achieved in India by application of the Alphabet Strategy with that of outpatient care in UK.

Materials and Methods: 100 outpatient records in an outpatient setting were assessed for the quality of diabetes care based on the Alphabet Strategy. The Alphabet Strategy of diabetes care was slightly modified by using Fasting and post prandial plasma glucose instead of HbA1c and proteinuria for microalbuminuria to suit Indian economic standards. A prospective audit was done using this modified strategy on further 100 patients with diabetes. The template based on the strategy prompted the medical team to verify the previous year outpatient record and deliver the essential components of diabetes care if needed. Data were collected and compared against 100 case notes randomly picked at the UK Diabetes centre, where the strategy is in use for 6 years.

Results: With the implementation of the modified Alphabet Strategy, the recording of BMI, Blood pressure and smoking at the Indian centre was 99% before and after vs. 100% in UK; the recording of cessation advice was 100% in the both centre. The recording of total cholesterol was 60% before and 99% after (p<0.0001) vs. 97% in UK. The recording of lipid profile also improved from 10% to 64% (p<0.0001) vs. 97% in UK. The record of creatinine improved from 5% to 49% (p<0.0001) vs. 99% in UK and the record of proteinuria improved from 48% to 93% (p<0.0001) after applying the template (56% in UK). 41% of the patients had both fasting and postprandial glucose which improved to 97% after implementation of strategy (HbA1c measured 100% in UK). The Indian centre achieved 100% on recording the status of eye and feet examination during the audit which was 98% and 95% respectively before.

The usage of guardian drugs in India before and after the strategy compared to UK was: Aspirin 6% vs.71% (UK 71%), ACE inhibitors or ARB 7% vs. 57% (UK 75%) and Statin 5% vs. 38% (UK 73%) and all these were statistically significant. 20% of the Indian patients were on all 3 compared to 52% in UK. The overall score, based on the GMS contract system of UK, improved from 45 to 61 in India compared to 76 in UK out of a maximum possible 99.

Conclusion: The Alphabet strategy of diabetes care can potentially be easily and effectively adopted for any setup of diabetes care, with a few modifications, to suit any healthcare economy. This strategy is an useful model that ensures that comparable standards of diabetes care are achieved in a cost effective way.

0818

Glycaemic control after initiation of oral antidiabetic therapies

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Background and Aims: Currently available oral antidiabetic agents (OADs) are reportedly unable to sustain good glycemic control over time, often requiring the addition of new therapies in order to maintain glycemic control. The objective of this study was to analyze the duration of commonly used OAD therapies before loss of glycemic control (HBbA_{1c}≥7.0%) occurs. Secondary analysis assessed the time taken before therapy intensification occurred in subjects with type 2 diabetes (T2D) who were treated with OADs.
Materials and Methods: A retrospective analysis was performed using eligibility, laboratory, medical and pharmacy claims data from a large US health care organization. Subjects were included if they had a claim for T2D, no claim for T1D and at least one HbA_{1c} measure recorded 90 days±the index date (date of first OAD claim) during the period 07/01/01 through 06/30/02. Study subjects were grouped as monotherapy cohorts (SU, MET, TZD) and combination therapy cohorts (SU+MET, SU+TZD, MET +TZD, SU+MET+TZD) irrespective of treatment order and based on prescription claim history for OADs during the baseline period and within 90 days of index date in the follow-up period. Subjects were followed for a minimum of 24 months and a maximum of 42 months. Demographic and clinical variables (comorbid conditions, baseline HbA_{1c}, creatinine, and cholesterol values), and prescribing clinician specialty were assessed for each subject. Cox proportional hazards model was used to compare time to reach HbA_{1c}≥7.0% and time to therapy intensification among the different treatment cohorts.
Results: Of the 6,131 identified subjects, 69% had an HbA_{1c}≥7.0% in the follow-up period. 82% of subjects in the SU+MET+TZD cohort had an HbA_{1c}≥7.0% in the follow-up and had the shortest period of time (323 days) until HbA_{1c} became ≥ 7.0%. 49% subjects in the TZD monotherapy cohort had an HbA_{1c}≥7.0% in the follow-up and had the longest time (418 days) until HbA_{1c}≥7.0%. After controlling for demographic and clinical characteristics and clinician specialty, the Cox model revealed that the SU+MET and SU+MET+TZD cohorts were 16% and 31% more likely to reach an of HbA_{1c}≥7.0% compared to the SU monotherapy cohort. In contrast, patients in the MET and TZD monotherapy cohorts were 18% and 38% less likely reach an HbA_{1c}≥7.0% compared to the SU monotherapy cohort. On average, subjects remained on their current therapy for 499.1±294.3 days before a new therapy was added. A higher percentage of subjects in the SU monotherapy cohort (34%) had an intensification of therapy while the percentage was smallest in the SU+MET +TZD combination cohort. Subjects in the SU+MET cohort had the longest time to therapy intensification (531.4±300.6 days). Time to therapy intensification was shortest in the TZD cohort (444.6±299.1 days). After controlling for baseline demographic and clinical variables and clinician specialty, the results of the Cox model indicate that the hazard of therapy intensification was greater in the MET (58%), TZD (31%) and SU+MET (22%) cohorts than in the SU cohort.
Conclusion: Results of this analysis suggest that about two-thirds of the study population on OADs did not have their HbA_{1c} under good control. Although there were differences between treatment cohorts, subjects in all OAD cohorts remained on therapy an average of 1.4 years before intensification of therapy regimen occurred.
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0819

The importance of weight changes on the economic burden of type 2 diabetes treatment

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Background and Aims: Obesity and type 2 diabetes are considered as epidemics of the modern societies. In addition, both conditions are closely related to each other and their prevalence increase in parallel. It is well known that these two conditions raise a heavy economic burden on the health care systems of all societies. The well-documented need for a decent glycemic control of type 2 diabetic patients is often accomplished with the use of multiple antidiabetic medications. On the other hand, weight management remains a cornerstone on the overall type 2 diabetes treatment and is often neglected due to the chronic strain of both patients and health care proffessionals.
Aim of this study was to examine the influence of weight loss change on the economic cost of type 2 diabetes treatment with oral hypoglycemic medications and/or insulin.
Subjects and Methods: The files of 1112 consecutive type 2 diabetic patients who referred to the diabetes outpatient clinic of our hospital form January 2000 to December 2003 were reviewed. Only those patients who have been

followed regularly in our clinic (having at least 3 visits per year for a minimum of 24 months) were included. All patients attending the clinic were given dietary advise regularly by specialized dieticians. The cost of treatment was measured in Euros, using the 01-01-2006 official greek market prices (as indicated in every medication's label) of all licenced antidiabetic medications in Greece. Skewed data were log-transormed to improve normality for statistical testing.

Results: Data from 264 patients (115 men [43.6%], mean age 61.8±10.8 years, mean duration of diabetes 9.0± 8.2 years) who fullfilled the inclusion criteria were analyzed. The mean duration of follow-up was 42.4± 16.6 months and during that period, mean HbA_1c decreased by 1.47% (from 8.54±2.0% to 7.07±1.13%). At the same time, body mass index (BMI) increased by 0.75Kg/m^2 (from 29.36±4.97 kg/m^2 to 30.11±5.13Kg/m^2). At the end of the study period, the mean cost of antidiabetic drug treatment per patient/month was 28.22±26.40 Euros. In multivariate linear regression analysis, after adjustment for age, gender, duration of diabetes, BMI, type of antidiabetic treatment, drug choice and HbA_1c achieved, the final cost of antidiabetic medication was positively associated to the degree of weight gain (standardized β-regression coefficient: 0.181, $P<0.0001$, $R^2=51.1$).

Conclusion: Weight loss, or even less weight gain, is associated with a reduction of the economic burden of antidiabetic medications used for glycemic control of type 2 diabetic patients.

0820

Achievement of clinical targets for type 2 diabetes is variable accross primary care populations in England, Scotland and Wales

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Background and Aims: The GMS contract has encouraged intensification of glycaemic lowering therapy, blood pressure lowering and treatment to cholesterol targets in the Type 2 diabetes population in the UK. Here we examine target achievement in typical English, Welsh and Scottish primary care populations

Materials and Methods: Data was exported from General Practices who consented to electronic audit in pooled anonomised format to a central server. Results presented represent latest patient data exported in the period March-October 2005.HbA1c results were available on 57427 English patients, 3412 Welsh patients and 23694 Scottish patients.

Results: Average HbA1cs were 7.84% England, 7.44% Wales and 7.86% in Scotland. Patients in Wales displayed the best HbA1c control, odds ratio for meeting 7% target: 1.33 vs England, 1.64 vs Scotland ($p<0.0001$). There was a similar trend for worse glycaemic control for monotherapy patients in Scotland, odds ratio 1.17 for 7% HbA1c target achievement England vs Scotland, $p<0.0001$. BP target achievement was also better in Wales, odds ratio for 140/80 target achievement 1.12 Wales vs England, 1.89 Wales vs Scotland, $p<0.0001$. Total cholesterol target <5 was met by a significantly higher number of Scottish patients, odds ratio Scotland vs England 1.22, Scotland vs Wales 1.34.

Conclusion: Whilst no correction is made for duration of diabetes or population age this is not likely to have impacted significantly on the results of this audit. There is clear evidence of differential control of blood glucose, blood pressure and cholesterol in the Type 2 diabetes population between the three countries and it is important all patients are managed aggresively to treatment targets. This is particularly important for patients managed with oral monotherapy for blood glucose lowering. As the largest treated patient group they represent an important opportunity for early intervention.

0821

Health related quality of life of body mass index for people with type 2 diabetes in the general adult population in England

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Background and Aims: The risk of developing cardiovascular and other life threatening diseases is strongly linked to increased body weight. This is particularly relevant to people with type 2 diabetes (T2D) in whom over 80% are overweight or obese. However, limited data exist on the impact on Health Related Quality of Life (HRQOL) of Body Mass Index (BMI) for people with type 2 diabetes.
The aim of this study was to characterize the impact of BMI on HRQOL for people with and without T2D in England.

Materials and Methods: Data from the Health Survey for England 2003 (HSE03) commissioned by the Department of Health was evaluated. Data was collected during the period January 2003 to March 2004. HSE03 consisted of a stratified random sample designed to provide data about the general population living in private households in England. Observations for 13,233 non-diabetic subjects and 461 patients with T2D (age≥18 years) were included in the analyses.

HSE03 involved a questionnaire-based interview followed by a nurse visit, where measurements and blood samples were taken. HRQOL was measured using the EQ-5D questionnaire. EQ-5D is a generic measure of HRQOL that generates a single index score based on a descriptive system that defines health in terms of 5 dimensions (mobility, self-care, usual activities, pain/discomfort and anxiety/depression). An individual who had no problems in any domain would score 1, that is, a health state of perfect health. Death is regarded as zero.

Overall HRQOL differences were investigated using t-tests. Analyses of variance (ANOVA) were used to investigate relations between EQ-5D scores and different categories of BMI (underweight (<18.5), normal (18.5−<25), overweight (25−<30), obese (30−<40) and extremely obese (≥40).

Results: The overall HRQOL was significantly lower for people with T2D compared to non-diabetic subjects, with EQ-5D scores of 0.795 versus 0.867, respectively ($p<0.01$). An ANOVA showed that the non-diabetic obese subgroup (BMI≥30) had a significant reduced HRQOL compared to others ($p<0.05$). Similarly, the HRQOL for people with T2D was significantly ($p<0.05$) lower for the morbidly obese (BMI≥40) compared to people in the normal weight band (BMI of 18.5−<25). The HRQOL was lower for people with T2D across all BMI categories compared to non-diabetics; however, due to sample size some of these findings were not significant.

Conclusion: These findings suggest that obesity in the general adult population in England has a significant impact on HRQOL. The impact of obesity on HRQOL is particularly evident in people with T2D who are further burdened by the impact of their disease.

Supported by Novo Nordisk A/S

PS 65

Liver disease and transplantation-induced diabetes

0822

Metabolic syndrome in long-term survivors of haematopoietic stem cell transplantation: evidence for a role of TNF-α

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Background and Aims: The success of haematopoietic stem cell transplantation (HSCT) has resulted in a large number of patients becoming long-term survivors. In adults the late effects of HSCT include graft versus host disease and its sequelae, effects of long-term immune deficiency, end-organ damage, development of secondary malignancies, and endocrine disorders, including gonadal function and fertility. Until recently, the metabolic disorders following HSCT in adults were unknown.

The purpose of this study is to assess the prevalence of metabolic syndrome (MS) among HSCT long-term survivors and to investigate the potential clinical and biological risk factors for cardiovascular disease (CVD) in this group of patients.

Materials and Methods: The study was carried out in 50 out-patients (27 men and 23 women, mean age 45.4± 9.7 yrs) of the Bone Marrow Transplantation Centre, defined long-term survivors because continuous complete remission lasted at least five years. Clinical characteristics were assessed by data collection concerning waist circumference, blood pressure, and current use of medications for hypertension and/or type 2 diabetes. Blood samples were taken from patients after an overnight fast to evaluate serum concentration of glucose, total and HDL cholesterol, triglycerides, insulin, adiponectin, resistin, leptin, and TNF-α. Differences between the means in the groups were evaluated using paired t-test in the case of normal distribution of data sets and Mann-Whitney Rank Sum test when at least in one of the data sets the normal distribution was excluded.

Results: The MS, as defined by the International Diabetes Federation (IDF) criteria, was present in 20 percent of the patients. This subgroup was characterized by elevated serum TNF-α concentrations when compared with the other patients (11.8±3.3 *vs* 9.6±3.6 pg/mL; $p<0.05$). No difference was found in the prevalence of autologous *vs* allogeneic HSCT.

Using the TNF-α median observed in the patients with MS (11 pg/mL) as a cut-off value for all the patients, two groups were obtained without significant differences in clinical characteristics. Serum insulin (19.9±3.3 *vs* 12.8± 1.1 mIU/mL; $p<0.01$), leptin (26.1±8.5 *vs* 14.9±1.9 ng/ml; $p<0.001$), resistin (8.9±1.7 *vs* 5.7±0.5 ng/mL; $p<0.01$), and triglycerides (164.5±18.6 *vs* 101.5±10.2 mg/dL; $p<0.001$) were significantly higher in the patients with TNF-α≥11 pg/mL than in the others. Moreover, serum HDL-cholesterol concentrations were significantly lower in the first investigated group (54.0±3.5 *vs* 67.5±4.0 mg/dL; $p<0.01$).

Conclusion: The prevalence of MS is high in patients who have had HSCT in adulthood. TNF-α increase is detectable either in patients with MS or in patients with some of the biochemical abnormalities observed in MS, i.e. lipid profile and serum concentrations of insulin, leptin, and resistin. So, the proinflammatory cytokine TNF-α, implicated in insulin

resistance, could be a biological marker of the inflammatory state predisposing to MS. In conclusion, MS could be considered an adverse long-term effects of HSCT, affecting the quality of life of patients surviving the procedure and making this growing population prone to CVD.

0823

Carbohydrate metabolism disorders in patients after renal transplantation

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Background and Aims: Post-transplantation diabetes mellitus is a complication occurring relatively often after renal transplantation. It significantly decreases prognosis as to the patient's survival and graft functioning. The aim of the study was to analyze the carbohydrate metabolism disorders in patients with chronic renal failure treated by renal transplantation and to estimate diabetogenic effect of immunosuppressive medications.
Materials and Methods: Ninety one patients after renal transplantation were investigated (55 men and 36 women). Average age of the study population was 48, with the mean weight of 76.9 kg and the mean post-transplantation period was about 3.8 years. Patients had been dialysed before transplantation for mean time of 28.7 months. Acute graft rejection were observed in 21 patients (23%). The metylprednisolon pulses (Solu-Medrol) were administered in 22 patients. The average creatinine concentration at the time of screening was 1.61 mg/dl, BUN - 27,41 mg/dl, total cholesterol concentration − 211.7 mg/dl, triglycerides concentration − 174 mg/dl. The mean systolic blood pressure was 117.5 mmHg and diastolic blood pressure − 82.3 mmHg in analyzed group. The patients were treated with following therapeutic regimen: cyclosporin/tacrolimus combined with azatioprine/mycophenolate mophetil (MMF) and prednisone. The standard oral glucose tolerance test (OGTT) and HbA_{1C} level were performed in the tested group.
Results: The following carbohydrate disorders were observed in the analyzed group: normal glucose tolerance (NGT) - in 31 patients (34.4%), impaired fasting glucose (IFG) - in 21 patients (23.3%), impaired glucose intolerance (IGT) - in 18 patients (20%), post-transplantation diabetes mellitus (PTDM) - in 15 (16.4%), diabetes as the cause of chronic renal failure - in 2 (2.2%), and diabetes of type 2 diagnosed before transplantation and not being the cause of renal failure - in 3 patients (3.3%). The significantly higher percentage of PTDM was found in patients taking tacrolimus (50%) as compared with 16% of patients treated with cyclosporin The average HbA_{1C} concentration was the highest in patients treated with tacrolimus combined with MMF and prednisone (6.69%). In patients treated with azatioprine and prednisone the HbA_{1C} level was 6.38%. In patients treated with cyclosporin the HbA_{1C} level was 6.26% although not reaching statistical significance.
Conclusion: It is necessary to control the glycemia level carefully in the renal graft recipients so as to make an early diabetes diagnosis and to begin the treatment as soon as possible. Post-transplantation diabetes was more frequently found in the group treated with tacrolimus.

0824

New-onset diabetes impaired fasting glucose after renal transplantation: results of a prospective, randomised trial comparing cyclosporine versus tacrolimus

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Background and Aims: New-onset diabetes mellitus (NODM) and other categories of glucose intolerance (OCGI) after solid organ transplantation are associated with increased morbidity and mortality. Among several risk factors predisposing to NODM or OCGI, the type of immunosuppression plays a major role. Here we describe the results of DIRECT, a 6-month, open-label, randomized, prospective multicenter study which used the ADA/WHO criteria to define glucose abnormalities, and compared cyclosporine versus tacrolimus in kidney graft recipients.
Materials and Methods: *De novo* renal transplant patients were stratified pre-transplant as (1) diabetic (2) non-diabetic/Caucasian or (3) non-diabetic/non-Caucasian, and were randomized to cyclosporine microemulsion (CsA-ME) with C_2 monitoring or tacrolimus. All patients received mycophenolic acid therapy, steroids, and basiliximab. The primary safety endpoint was a composite of NODM or impaired fasting glucose (IFG) at 6 months in patients who were non-diabetic at baseline; the primary efficacy endpoint was a composite of biopsy-proven acute rejection (BPAR), graft loss or death at 6 months in all patients.

Results: The population analyzed comprised 682 patients (336 CsA-ME, 346 tacrolimus): 115 were diabetic at baseline, 483 were non-diabetic/Caucasian and 84 were non-diabetic/non-Caucasian. Patient characteristics, diabetes risk factors and steroid doses were similar between treatment groups. NODM or IFG occurred in 73 CsA-ME patients (26.0%) and 96 tacrolimus patients (33.6%, p=0.046) at 6 months. In the Caucasian and non-Caucasian subpopulations, the 6-month incidence was 24.8% and 32.6% with CsA-ME and 32.2% and 39.0% with tacrolimus, respectively. At month 6, 12.5% of CsA-ME patients and 18.0% of tacrolimus patients who were non-diabetic at baseline required hypoglycemic treatment (p<0.05). The incidence of BPAR, graft loss or death was 12.8% with CsA-ME and 9.8% with tacrolimus (p=0.211). Blood pressure and renal function were similar. An increase in both median LDL- and HDL-cholesterol occurred in the CsA-ME group (LDL+0.42 mmol/L, p<0.0001; HDL +0.23 mmol/L, p<0.0001). The ratio of total cholesterol/HDL remained unchanged (4.03 vs 3.98 at month 6, p=0.43). Adverse events were similar between groups.
Conclusion: The incidence of NODM or IFG at 6 months after renal transplantation is significantly lower with CsA-ME than tacrolimus with no difference in terms of efficacy.
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0825

Intermediate clinical phenotype of diabetes in HCV patients

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Background and Aims: There is mounting evidence to indicate the genuine link between diabetes mellitus (DM) and hepatitis C virus infection (HCV). In the majority of studies diabetes was classified as type 2. Apparently, both insulin resistance (IR) and islet cell dysfunction have been reported to contribute to the development of diabetes in HCV patients. Moreover, the predictors for this association such as older age, obesity, family history, Black ethnicity are well accepted as the classical risk factors for type 2 diabetes in the general population. However, several questions concerning the true character of diabetes in this group of patients must be underlined. Direct cytopathic effect of HCV at the islet cell level as well as viral-induced IR may be pathogenic mechanisms causing DM in HCV patients. Thus we propose that the diagnosis of type 2 diabetes in this special group of patients should not be so straightforward. Our aim was to establish the clinical phenotype of diabetes in non-cirrhotic HCV patients.
Materials and Methods: 38 non-cirrhotic HCV (positive anti-HCV antibodies and HCV-RNA) diabetic patients (23 men and 15 women, aged: 47.5±11.8, duration of diabetes: 5.1±4.4 years, HCV infection: 4.1±3.0 years; HbA1c 8.0±2.3%) were compared with patients with type 2 (n=25, 18 men and 7 women, aged: 49.6±6.4, duration of diabetes: 9.0±5.9 years; HbA1c 8.3±1.4%) and type 1 (n=32, 19 men and 13 women, aged: 44.8±7.1, diabetes: 16.1±10.6 years; HbA1c 8.2±1.3%) and with non-diabetic non-cirrhotic HCV patients (n=23, 14 men and 9 women, aged: 41.0±12.4). In all the study groups selected metabolic variables such as systolic and diastolic blood pressure, blood lipids and body-mass-index (BMI) were measured.
Results: HCV diabetic patients presented with intermediate clinical phenotype in comparison with both type 1 and type 2 diabetic controls (Table).

	HCV diabetes	Diabetes type 2	Diabetes type 1	HCV
BMI (kg/m^2)	26.5±4.8	30.9±6.3*	24.0±3.4$^{\#}$	25.0±3.2
SBP (mmHg)	133.9±14.0	142.9±25.6*	128.8±17.0$^{\#}$	123.8±16.6$^{\#}$
DBP(mmHg)	84.4±10.2	88.1±16.0*	82.2±11.6$^{\#}$	79.5±8.9$^{\#}$
TC (mmol/l)	3.5±0.7	4.7±0.7*	4.3±0.8*	1.7±0.5
LDL-C (mmol/l)	1.9±0.5	2.7±0.8*	2.3±0.7*	2.4±0.6
HDL-C (mmol/l)	1.0±0.3	1.0±0.2	1.4±0.4* $^{\#}$	1.1±0.1
TG (mmol/l)	1.4±0.8	2.6±1.9*	1.2±0.6$^{\#}$	0.8±0.1

*p<0.05 vs HCV diabetes; $^{\#}$ p<0.05 vs diabetes type

Conclusion: The current study indicates that a distinct diabetic phenotype is associated with HCV infection. Therefore, it should be emphasized that the complex pathogenesis of diabetes in HCV patients together with the observed differences in the clinical phenotype cast doubt on the diagnosis of type 2 diabetes in that group of patients.

0826

High incidence of de novo diabetes mellitus type 1 during or after treatment with pegylated interferon in patients with chronic hepatitis C virus infection

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Background and Aims: Previous reports have shown the occurrence of diabetes mellitus (DM) during treatment with interferon in patients chronically infected with hepatitis C

virus (HCV). Both type 1 and type 2 DM have been described. We prospectively assessed the incidence of DM during or shortly after treatment with pegylated-interferon (PEG-IFN) and ribavirin (RBV) and the type of DM.

Material and Methods: After observing a first case of hyperglycaemia during PEG-IFN/RBV treatment in our hospital, blood glucose levels (PGL) of patients with HCV have been routinely assessed at all outpatient visits during and after treatment with PEG-IFN/RBV since January 2003. In those patients who developed DM, autoantibodies to glutamic acid decarboxylase (anti-GAD65), protein tyrosine phosphatase (anti-IA2) and pancreas islet cells (ICA) were investigated by standard immunoassays.

Results: Between January 2003 and October 2005, 150 patients were treated with PEG-IFN/RBV of which 8 developed hyperglycaemia (5,3%). Classical symptoms of DM were present in only 2 patients who both developed DM shortly after cessation of PEG-IFN/RBV. One of these patients has achieved a sustained virologic response (SVR). In all other patients, DM was diagnosed by elevated PGL, and symptoms of DM were either indistinguishable from side-effects provoked by PEG-IFN/RBV or not present. Retrospective analysis revealed that in 2 patients anti-GAD65 became positive after 11 and 22 weeks administration of PEG-IFN (table 1). One patient was positive for anti-GAD65 before treatment. Two patients tested weakly positive for anti-IA2 during treatment. One patient became positive for ICA during treatment. Finally, 2 of 8 patients achieved an SVR.

Conclusion: In our population the incidence of diabetes mellitus during therapy with PEG-IFN/RBV is higher than previously reported by others. Classical symptoms associated with DM may be masked by or mistaken for side-effects provoked by treatment with PEG-IFN/RBV. Most patients were identified by routine assessment of PGL. We therefore suggest routine assessment of PGL at all outpatient visits before, during and after PEG-IFN/RBV treatment for patients infected with HCV.

Autoantibodies before and during treatment with PEG-IFN

Patient	anti-GAD65 before	anti-GAD65 during	anti-IA2 before	anti-IA2 during	ICA before	ICA during
1	neg	neg	neg	neg	neg	neg
2	neg	neg	neg	weakly pos	neg	neg
3	neg	neg	neg	neg	neg	neg
4	weakly pos	positive	neg	neg	neg	neg
5	neg	neg	neg	neg	neg	neg
6	neg	positive	neg	neg	neg	neg
7	neg	positive	neg	neg	neg	neg
8	positive	positive	neg	weakly pos	weakly pos	positive

0827

Exenatide improved elevated hepatic alanine transaminase (ALT) and reduced weight in patients with type 2 diabetes mellitus

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Background and Aims: Type 2 diabetes mellitus (T2DM), obesity, and nonalcoholic fatty liver disease (NAFLD) are emerging public health concerns that often co-exist; with diabetes and obesity the two most important risk factors for NAFLD. In T2DM patients not achieving glycemic control with MET and/or SFU and enrolled in 30-wk, placebo-controlled, phase 3 trials followed by open-label extensions (63%M, age 57±10 y, wt 100±19 kg, BMI 34±6 kg/m^2, A1C 8.3±1.0% [mean±SD]), the incretin mimetic exenatide improved glycemic control, reduced body weight, and was generally well tolerated. We evaluated whether these patients had ALT changes that might indicate improved NAFLD.

Materials and Methods: A *post hoc* interim analysis of the liver biomarker ALT was performed, with a subanalysis by weight change quartiles at 2 years. Patients were stratified by normal baseline ALT values: ≤19 IU/L (F), ≤30 IU/L (M; Prati 2002). An alternate analysis was done using the older guideline: normal ≤37 IU/L (F),≤48 Üé/¥ (M).

Results: Patients completing 2 years on exenatide (n=283) had reduced A1C (−1.1±0.1%; p<0.05) and weight (−4.7±0.3 kg; p<0.05) from baseline (mean±SE). Patients with normal baseline ALT (n=132) had minimal ALT change:+1±1 IU/L from baseline 21±0.4 IU/L (NS) with −3.9±0.5 kg from baseline weight 99±2 kg. In contrast, patients with elevated ALT at baseline (n=151) had reductions in ALT: −11±1 IU/L from baseline 38±1 IU/L (p<0.05) with −5.3±0.5 kg from baseline weight 100±2 kg. Patients in the high baseline ALT (41±3 IU/L)/highest weight loss quartile had −18±4 IU/L ALT reduction and −13.5±0.7 kg weight change (n=38). Patients with elevated ALT at baseline tended to lose more weight than patients with normal ALT at baseline (p=0.04), however weight change was minimally correlated with baseline ALT (r=−0.09). Similar results were obtained for the ITT and alternate ALT-range analyses.

Conclusion: Exenatide-treated T2DM patients with elevated baseline ALT had ALT reductions, suggesting exenatide may improve NAFLD in this patient population.

0828

Effects of pioglitazone on liver function in triple oral therapy: a 42-week, placebo-controlled randomised study

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Background and Aims: Currently there are no safety data from randomised, double-blind studies that have examined the effects of triple oral anti-diabetic therapy (combination of metformin [MET], a thiazolidinedione [TZD] and a sulphonylurea [SU]) on liver function tests. Previous studies of pioglitazone (PIO) have demonstrated favourable effects on liver enzymes when either used as monotherapy or in combination with either MET or an SU. We evaluated the effect on liver function of adding in PIO to MET plus an SU in inadequately controlled, insulin-naïve patients with Type 2 diabetes.

Materials and Methods: This prospective, double-blind, placebo-controlled study randomised 299 patients with Type 2 diabetes previously treated with MET (≥1700 mg/day) and SU (maximal dose) for more than 3 months and HbA_{1c} between 7.0% and 9.5%. After a 4-week run-in, patients received either PIO 30 mg once daily or placebo (PBO). After 3 months, if HbA_{1c}>6.5%, PIO or PBO was up-titrated to 45 mg for the remaining 4 months. Liver function tests were conducted at baseline and at final visit (7 months) for alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutamyltransferase (GGT).

Results: Mean changes (SD) from baseline at Month 7 for ALT (U/L) were −6.8±11.6 with PIO and −0.2±10.5 with PBO, for AST (U/L) −1.2±6.0 with PIO and −0.7±6.8 with PBO and for GGT (U/L) −9.5±20.1 with PIO and 3.8±19.9 with PBO. Of the 37 patients with high baseline ALT values in the PIO group, 48.6% (18/37) were normalised at Month 7 vs. 40.7% (11/27) in the PBO group. Of the 13 PIO patients with elevated baseline AST, 76.9% (10/13) were normalised at Month 7 vs. and 66.7% (6/9) with PBO. Of the 43 patients in the PIO group with elevated GGT at baseline, 48.8% (21/43) were normalised at Month 7 vs. 10.8% (4/37) in the PBO group.

Conclusion: Triple oral anti-diabetic therapy with PIO plus high doses of MET and SU was associated with liver function improvement after 7 months. There was no evidence of hepatotoxicity, consistent with previous findings with pioglitazone.

Shift in the % of patients with high or normal baseline values to high or normal values at Month 7

	Pioglitazone		Placebo	
Baseline value:	**High**	**Normal**	**High**	**Normal**
ALT	n=37	n=97	n=27	n=109
High (>30/40 U/L)	51.4	5.2	59.3	11.0
Normal	48.6	94.8	40.7	89.0
AST	n=13	n=121	n=9	n=127
High (>32/38 U/L)	23.1	0.8	33.3	1.6
Normal	76.9	99.2	66.7	98.4
GGT	n=43	n=92	n=37	n=99
High (>32/49 U/L)	51.2	3.3	89.2	8.1
Normal	48.8	96.7	10.8	91.9

'High' values are female/male; n=number of patients with data at baseline and Month 7.

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0829

Enalapril attenuates hepatocytes apoptosis in diabetic rats: role of oxidative stress

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Background and Aims: Oxidative stress is involved in both the pathogenesis and complications of diabetes. It has been shown that ACE inhibitors can slow reduce oxidative stress and tissue injury in the streptozotocin-induced diabetic rats. The aim of the present study was to determine the role of the enalapril treatment in the reduction of hepatocytes apoptosis of diabetic rats.

Materials and Methods: The rats were divided into the following groups: STZ group, received streptozotocin (65 mg/kg, single intraperitoneal dose); STZ-ENL group that received both streptozotocin injection and enalapril (20 mg/L drinking water), and Control (intraperitoneal saline). Five months after streptozotocin injection, rats were sacrificed and the liver samples were obtained for evaluation of hepatocytes apoptosis index and measurement of glutathione concentration and antioxidant enzymes activities for assessment of oxidative stress.

Results: Five months after injection of streptozotocin, the number of apoptotic hepatocytes was increased significantly in diabetic rats (3.12±0.21%) compared to control animals (0.91±0.13%) (P<0.001). Treatment of diabetic rats with enalapril significantly reduced the number of apoptotic

hepatocytes in STZ-ENL group (2.04±0.19%), compared to STZ group (P<0.01); however percentage of apoptotic hepatocytes was still higher than control group (P<0.01). Streptozotocin-induced diabetes was associated with a decrease in hepatic glutathione concentrations (6.95±0.24 vs. 4.78±0.35, P<0.001), which was significantly attenuated following chronic treatment with enalapril (5.41±0.28) (P<0.01 vs. Control and P<0.05 vs. STZ). Liver catalase, glutathione peroxidase, and superoxide dismutase activies were significantly reduced in rats of STZ group compared to control. Chronic treatment with enalapril raised the levels of hepatic antioxidant enzyme activities in diabetic animals significantly to levels that were not different from control values.

Conclusion: For the first time, our findings demonstrate that chronic enalapril treatment is protective against hepatic damage in streptozotocin-induced diabetic rats. These results suggest that the protective action of enalapril might be mediated, at least in part, by its effect on tissue oxidant/antioxidant balance.

0830

Associates of the change in liver fat content after weight loss

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is a common manifestation of obesity and type 2 diabetes. Known predictors of NAFLD include serum ALT, visceral obesity and insulin resistance. NAFLD improves following weight loss. The degree of resolution of NAFLD following weight loss is not routinely assessed. The aims of this study were to identify simple anthropometric or biochemical correlates of liver fat content (LFAT) at baseline and the correlates of the change in LFAT following weight loss in a group of morbidly obese (BMI 31–52 kg/m^2) women. To assess any effect of the type of weight loss intervention on correlates of change in LFAT, subjects were divided into a non-surgical arm utilising only a very low calorie diet (VLCD) or a surgical arm using laparoscopic adjustable gastric banding (LAGB). The duration of intervention was determined by the estimated time to achieve 8% weight loss.

Materials and Methods: LFAT and abdominal subcutaneous (SAT) and visceral adipose tissue volumes (VAT) were measured using magnetic resonance (MR) spectroscopy and MR imaging before (n=48) and, where possible, after 6 weeks of Optifast VLCD (n=14) or 3 months after LAGB (n=24). Anthropometry (body weight, BMI and waist circumference) and fasting blood tests (liver enzymes, free fatty acids, insulin, glucose, ferritin, leptin and adiponectin) were performed before and after each intervention. Insulin sensitivity was estimated by homeostasis model assessment (HOMA). Pearson's correlation coefficient was used to relate variables. P≤0.05 indicated statistical significance.

Results: LFAT content in both groups was similar at baseline (0.14±0.12 SD AU VLCD; 0.11±0.11 AU LAGB). Eleven patients in the VLCD arm and 15 patients in the LAGB arm had hepatic steatosis at baseline (LFAT> 0.05 AU). As expected, in the group as a whole at baseline, LFAT was best correlated to ALT (r=0.707, p<0.01), VAT (r=0.691, p<0.01) and HOMA (r=0.502, p<0.01). Mean ALT=29±14 U/L (normal <50 U/L). Neither plasma ferritin, leptin nor adiponectin related to LFAT at baseline. LFAT related to waist circumference at baseline (r=0.396, p=0.005) but not to weight or BMI. Similar relative weight loss occurred after VLCD (8±3%) and LAGB (9±4%). LFAT content reduced to 0.08±0.08 AU and 0.08±0.11 AU respectively after weight loss but the change was only significant in the VLCD group (p=0.016). After VLCD, the change in LFAT was best related to the change in body weight (r=0.728, p=0.003).The change in ALT was the only significant correlate of the change in LFAT after LAGB (r=0.573, p=0.003).

Conclusion: In the morbidly obese female, LFAT relates to visceral adiposity and insulin sensitivity and relates closely to "normal" levels of ALT. After 6 weeks VLCD intervention, the success of LFAT removal can be best gauged by assessing the patient's weight loss. The effect on LFAT of the more gradual weight loss induced by LAGB can be better assessed by the extent of change in ALT. The differing associates of change in LFAT following these two interventions may reflect differing daily caloric or fat intakes and the differing time periods.

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Hypoglycaemia

0831

The Arg16Gly polymorphism of the β_2-adrenergic receptor and impaired hypoglycaemia awareness in patients with type 1 diabetes

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Background and Aims: Reduced β-adrenergic sensitivity has been reported in type 1 diabetic patients with impaired hypoglycaemia awareness, presumably as a result of a desensitization process. Desensitization of the β_2-adrenergic receptor is in part determined by the Arg16Gly polymorphism at codon 16 of the gene encoding the β_2-adrenergic receptor. We tested the hypothesis that impaired hypoglycaemia awareness is more common among patients homozygous Gly16.
Materials and Methods: We studied the effect of the Arg16Gly polymorphism of the β_2-adrenergic receptor in 85 Caucasian patients with type 1 diabetes. All subjects were characterized for basic demographic and clinical parameters. Hypoglycaemia awareness status was assessed using a standardized and well-validated questionnaire.
Results: A total of 45 patients (53%) were homozygous Gly16, 8 patients (9%) were homozygous Arg16 and 32 patients (38%) were heterozygous Arg16Gly. Nineteen of the homozygous Gly16 patients (42%) were classified as having impaired hypoglycaemia awareness, compared to 2 (25%) and 5 (16%) of the patients who were homozygous Arg16 or heterozygous Arg16Gly, respectively (p=0.04). Patients who were homozygous Gly16 were 3 times more likely to have impaired hypoglycaemia awareness than patients without this polymorphism (OR 3.4; 95%CI 1.3–9.4; p=0.01).
Conclusion: The results of this study indicate that type 1 diabetic patients homozygous Gly16 for the β_2-adrenergic receptor are at increased risk for having impaired hypoglycaemia awareness. Future studies are required to confirm these data in larger and other populations.
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0832

Symptomatic episodes of hypoglycaemia recorded by CGMS in patients with type 1 diabetes

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Background and Aims: We analyzed symptomatic episodes of hypoglycaemia recorded by the Medtronic MiniMed Continuous Glucose Monitoring System (CGMS).
Materials and Methods: A cohort of 119 patients with type 1 diabetes (age 46 years+12 (mean+SD); duration of diabetes 21 years+12; HbA1c 8.5%+1.0; 86% on multiple injections; 35% women) participated in 6 days of continuous subcutaneous glucose monitoring with CGMS. Self-monitored blood glucose (SMBG) measured with Hemo-Cue 201+was used to calibrate the CGMS four times daily. Participants kept a diary of meals, insulin dose, and episodes of symptomatic hypoglycaemia.
Results: A total of 2.6 symptomatic episodes/patient/week were reported by the patients: 2.3 (89%) in the daytime and 0.3 (11%) in the night (p<0.001). Episodes were less frequent in the night (11%) compared to mornings (30%), afternoons (34%) and evenings (25%) (p<0.001). One hundred and sixtyfive (63%) symptomatic episodes were accompanied by SMBG. The median values of SMBG and CGMS at these episodes were 2.7 mM (range 0.8–8.0) and 3.6 mM (2.2–11.9) (p<0.001). In 15% of the episodes a blood glucose concentration>3.5 mM was measured with SMBG versus 51% by CGMS. The most frequently reported reason for episodes was physical activity (36%). However, in 43% of the events no reason could be given. In the diaries, household chores were the most frequently self-reported activity during episodes.
Conclusion: In conclusion, most episodes of symptomatic hypoglycaemia occur in the daytime. The variation in blood glucose is wide whether measured by SMBG or CGMS. Physical activity is the most frequent reason for symptomatic episodes, however most events were unexplained.

0833

Effects of amino-acid stimulation on glucagon secretion in non-diabetic and type 1 diabetic subjects during hyperinsulinaemic eu-, hypo and post-hypoglycaemic hyperglycaemia

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Background and Aims: Response of glucagon (G) to hypoglycemia (H), is usually lost in type 1 diabetes mellitus (T1 DM). Aim of the present study was to establish if responses of G in T1 DM are selectively defective during H as compared to eu- and hyperglycemia.
Materials and Methods: We thus compared responses of G to alanine (A), an amino-acid (AA) known to stimulate G secretion, in a group of T1 DM subjects with those of non-diabetic (N-DS) during hyperinsulinemic eu-hypo- and hyperglycemic clamps. We studied 8 T1 DM (age 30± 11 yrs, BMI 23.4±4.3 kg/m^2, HbA_{1c} 6.9±0.9%) and 6 N-DS on 3 different, randomized, occasions for 370 min. On 2 occasions, i.v. l-A was infused either at the rate of 5.6 (low dose LDA) or 28 (high dose HDA) μmol/kg/min from −120 to 250 min. On a third occasion, saline (S) was infused throughout. In all studies, insulin at the rate of 0.5 mU/Kg/min was infused from 0 to 250 min. Variable glucose was infused to clamp plasma glucose (PG) at 2.8 mmol/l from 90 to 130 min, to restore euglycemia (5.5 mmol/l) from 150 to190 min, and to produce hyperglycemia (10 mmol/l) from 210 to 250 min.

Results: PG and insulin concentrations did not differ among studies (p>0.2). Plasma A increased similarly in T1 DM and N-DS. Glucose infusion rates (GIR) at clamped H were different in T1 DM: HDA (0.7±0.3), LDA (2.02±0.5) and S (3.03±0.6 mg/kg/min) (p<0.05). In N-DS, GIR was lower after HDA (0.9±0.2) as compared to LDA (1.69±0.4) and S (1.63±0.5) (p<0.05). In S, G increased in N-DS (121±23 ng/l) whereas it did not in T1 DM (51±5.6). G increased further after LDA (177±29) and HDA (228±36) in N-DS, but only after HDA (111±11) in T1 DM. G decreased after recovering euglycemia and following hyperglycemia both in N-DS and T1 DM.
Conclusion: In T1 DM, deficient response of G to H can be recovered by increasing plasma AA concentration. Nevertheless G response remains reduced in T1 DM as compared to N-DS. AA-induced secretion of G to H is largely suppressed by restoration of euglycemia and induction of hyperglycemia.
Supported by JDRF

0834

Subcutaneous infusion of very low dose-glucagon averts insulin induced hypoglycaemia in patients with type 1 diabetes mellitus

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Background and Aims: Insulin intensification to achieve optimal glycemic control and prevent microvascular complications of type 1 diabetes mellitus (t1DM) is associated with increased rates of hypoglycemia, the critical factor limiting optimized glycemic control. We studied the effects of continuous subcutaneous (SC) infusion of Very Low Dose-Glucagon (VLDG) (doses of <16 ng/kg/min) in patients undergoing insulin intensification in order to induce hypoglycemia.
Materials and Methods: Patients with the following baseline characteristics were enrolled: t1DM of>10 years duration, use of insulin pump therapy, BMI <29 kg/m^2 and HbA1c level <8.0%. After overnight fasting, patients underwent acute insulin intensification in increments of 50% every 90 minutes (up to 300% of basal insulin rates) to induce hypoglycemia (blood glucose (BG) <50 mg/dL). Under the same conditions of insulin intensification for a given patient, VLDG was then administered at doses of 4, 8, 12 or 16 ng/kg/min for up to 9 hours or until hypoglycemia was reached.
Results: Plasma glucagon levels reached peak concentrations within 1 hour of initiating the infusion and remained near peak levels for the infusion duration, returning to basal levels <1 hour after infusion cessation. VLDG administration induced an initial rise in BG levels that generally peaked within 1 to 2 hours and then declined. All VLDG doses studied either delayed or completely averted the onset of the insulin-induced hypoglycemia in a dose-dependent fashion; however these effects did not reach statistical significance.

VLDG Dose ng/kg/min	Mean Plasma Glucagon* (pg/mL)	Peak Blood Glucose (mg/dL)	Time to BG <70 mg/dL (in hours)	Time to BG <60 mg/dL (in hours)	Time to BG <50 mg/dL (in hours)
0 (N=14)	56	122	5.0	5.6	6.2
4 (N=6)	58	139.2	5.9	6.3	7.2
8 (N=9)	90	207	7.1	7.4	7.8
12 (N=9)	104	214	7.0	7.1	7.5
16 (N=8)	124	217	7.1	7.4	7.9

*reference range in non-diabetic individuals 50–150 pg/mL

Conclusion: In summary, continuous SC infusion of VLDG is well tolerated, results in a moderate increase in both glucose and glucagon levels, and is able to avert or delay the onset of hypoglycemia induced acutely by increasing basal insulin by 50 to 300%. VLDG will be studied further as a therapeutic intervention to avert hypoglycemia in association with insulin intensification.

0835

PREDICTIVE™: A global, prospective, observational study to evaluate insulin detemir treatment in type 1 and 2 diabetes - baseline characteristics and predictors of hypoglycaemia in the European cohort*

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Background and Aims: Observational studies are valuable in confirming clinical trial outcomes in clinical practice. The objective of PREDICTIVE™ (Predictable Results and Experience in Diabetes through Intensification and Control to Target: An International Variability Evaluation) - one of the largest observational studies in diabetes to date - is to assess the safety and efficacy of insulin detemir in a real-world setting. The primary endpoint is incidence of serious

adverse drug reactions, including major hypoglycaemia. Hypoglycaemia, traditionally associated with insulin therapy, is a key concern for patients. Secondary endpoints are change in: glycosylated haemoglobin A1c (HbA_{1c}); weight; fasting blood glucose (FBG); within-subject FBG variability; and incidence of minor hypoglycaemia. Here we report baseline patient characteristics from the European cohort and, in insulin-treated patients, assess the association of hypoglycaemia with baseline parameters.

Materials and Methods: A total of 19,911 patients (15,919 insulin-treated, 3,529 OADs only and 463 treatment naïve), with type 1 or 2 diabetes and requiring basal insulin, were prescribed insulin detemir and will be followed up for 12, 26 and/or 52 weeks. Demography and efficacy parameters were collected from patient records. Hypoglycaemic episodes were collected from patient records or recall in the 4 weeks before initiation of insulin detemir, and negative binomial distribution was used for hypoglycaemic modelling.

Results: Baseline characteristics are shown in the table. The incidence of hypoglycaemia in type 1 and 2 insulin-treated patients was positively correlated with duration of diabetes, total number of insulin injections and FBG variability ($P<0.05$ for all). The incidence of hypoglycaemia was inversely related to HbA_{1c}, FBG and body mass index (BMI; $P<0.05$ for all). Female patients had a higher incidence of hypoglycaemia than males.

Table: Baseline demographic, safety and efficacy measures

Parameter	Type 1 (n=7,067)	Type 2 (n=12,720)
Gender M/F, % patients	49.3/50.7	47.1/52.9
Mean age, years±SD	41.7±16.8	60.8±10.9
Mean diabetes duration, years±SD	16.5±12.5	11.1±7.5
Mean BMI, kg/m^2±SD	25.2±4.5	30.9±5.9
Mean HbA_{1c}, %±SD	8.3±1.7	8.5±1.6
Mean FBG, mmol/L±SD	9.7±3.4	10.6±3.3
Mean within-subject FBG variability (calculated as the SD of FBG), mmol/L±SD	2.8±1.9	1.6±1.3
Mean hypoglycaemic episodes, in the 4 weeks prior to study start		
All hypoglycaemic events±SD	3.7±6.3	0.7±2.4
Major hypoglycaemic events±SD	0.2±1.3	0.1±0.4
Pre-study/new therapy, % patients		
OAD only	1/0	27/0
Insulin	96.8/100	70.6/100

Conclusion: Baseline data from the PREDICTIVE™ European cohort show that insulin-treated patients enrolled in this study, in particular type 1 patients, were experiencing substantial hypoglycaemia. Based on observation of this large patient cohort, some interesting associations can be noted; the rate of hypoglycaemia increased with treatment intensification and degree of glycaemic control. Additionally, hypoglycaemia was positively correlated with duration of diabetes and FBG variability, and inversely related to BMI. Follow-up data from the PREDICTIVE™ study will provide valuable insights on the safety and efficacy of insulin detemir in the management of diabetes.

*Austria, Czech Rep., Denmark, Finland, Germany, Ireland, Israel, Netherlands, Sweden, Turkey and UK

0836

Sensitivity and specificity of CGMS for identifying clinially relevant symptomatic hypoglycaemia

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Background and Aims: Continuous Glucose monitoring (CGMS) is used in both the clinical and research setting to identify and to quantify hypoglycaemia. Interstitial glucose measured by CGMS differs physiologically from capillary glucose, so it is important to determine the optimal sensor glucose level which predicts clinically relevant symptomatic hypoglycaemia.

Materials and Methods: We analysed CGMS data from 381 patients (72% type 2 diabetic) who underwent a total of 10 days CGMS each over 2 periods separated by 8–10 months. During CGMS monitoring, they recorded capillary glucose readings during all symptomatic hypoglycaemia. We determined the corresponding sensor glucose value and analysed the relationship between sensor and capillary glucose during these events. We used ROC curve analysis to determine the sensitivity and specificity of different sensor glucose levels for identifying a capillary glucose of <3 mM.

Results: A total of 373 events of symptomatic hypoglycaemia were reported and corresponding sensor and capillary glucose were available for 234 of these. There were 7 episodes of severe hypoglycaemia with mean capillary glucose of 2.3 (range (1.2–3.5) mM). During these, corresponding sensor glucose was below the lower limit of detection (2.2 mM) in all but two episodes when the readings were 2.5 and 3.5 mM. Mean capillary glucose during 63 symptomatic events when the sensor glucose was below the lower limit of detection (2.2 mM) was 2.6 (range 1.2–3.9)mM. There were 173 symptomatic events with a sensor glucose above 2.2 mM and mean (sd) capillary and sensor glucose were 3.2 mM and 3.4 mM respectively.

Sensor glucose was a mean (sd) of 0.3(0.9) mM higher than capillary glucose and this difference was greater in type 2 than type 1 diabetic patients.
There were 121 events with a capillary glucose of <3 mM during which corresponding sensor glucose was also <3 mM in 73% and was <4 mM in 94% events. Sensitivity and specificity of different sensor glucose levels for identifying a capillary glucose <3 mM are shown in the table.
Conclusion: CGMS reliably identifies clinically relevant hypoglycaemia. For clinical use a sensor glucose of 3 mM appears optimal in identifying hypoglycaemia. However in the research setting, which requires greater specificity, we would recommend a cut off of 2.2 mM.

Sensitivity and Specificity of different sensor glucose cut offs for hypoglycaemia

Sensor glucose mM	Sensitivity %	Specificity %
4.0	93.7	22.4
3.5	82.9	40.8
3.0	64.9	68
2.5	46	80
2.2	39.6	86.4

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0837

Experience of hypoglycaemia is associated with lower quality of life (QoL) in German patients with type 2 diabetes on metformin (MF)+PPARγ agonist or MF+ sulphonylurea (SU) combination therapies

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Background and Aims: Hypoglycemia is associated with some glucose-lowering medications. Experience of hypoglycemia is expected to have a significant impact on patients' QoL and treatment compliance. This study was undertaken to evaluate the experience of hypoglycemia and its impact on QoL among diabetic patients treated with MF in combination with a PPARγ agonist or a SU in Germany.
Materials and Methods: This is an observational, cross sectional study conducted in 92 clinical centers in Germany in 2005. Patients (≥35 years old) with type 2 diabetes who had been treated with MF in combination with a PPARγ agonist or a SU for at least 6 months prior to study enrollment were included. Excluded were patients receiving insulin, treated for hepatitis or HIV, or having gestational diabetes. Case report forms were developed for collection of patients' medical record, experience of hypoglycemia, and general QoL. Both the EQ-5D VAS score and the un-weighted scoring rule for EQ-5D (100 point scale) were used to obtain QoL scores. Adjusted linear regression analysis was used to evaluate the effect of hypoglycemia on patients' QoL controlling for demographic and medical characteristics.
Results: Of the 392 patients included in the study, 29% (115) received MF in combination with a PPARγ agonist, and the remaining 277 metformin in combination with a SU. The average patient age was 62 years, with patients on MF+SU combination therapy being slightly older than those on MF+PPARγ agonist combination therapy (63 vs. 59 years, respectively). Forty three percent of the patients were female, and 45% had been diagnosed with type 2 diabetes for at least 7 years. Twenty seven percent (105) patients reported at least one episode of hypoglycemia during the previous 6 months. There were more patients on MF+SU combination therapy than MF+PPARγ agonist combination therapy (29% vs. 23%; $p>0.05$) that experienced hypoglycemia symptoms. Among patients with episodes of hypoglycemia, 103 (98%) experienced mild/moderate hypoglycemic symptoms, 54 (51%) had severe symptoms, with 51% reported experience of both mild/moderate and severe symptoms. More than half of those reporting hypoglycemic symptoms had at least 3 episodes during the previous 6 months. Relative to patients not reporting any hypoglycemic symptoms, patients with episodes of hypoglycemia reported lower EQ-5D VAS scores (71.7 vs. 65.5; $p=0.0072$) and lower un-weighted EQ-5D scores (82.4 vs. 71.5; $p=0.0003$). Hypoglycemia was associated with reductions in both EQ-5D VAS and the un-weighted EQ-5D scores (6.4, $p=0.0053$ and 11.5 $p<0.0001$ respectively) after adjusting for basic demographic and medical characteristics (age, gender, marital status, education, co-morbid conditions).
Conclusion: More than 27% of the study patients treated with metformin+PPARγ agonist or MF+SU combination therapies experienced symptoms of hypoglycemia. This study found that experience of symptoms of hypoglycemia is significantly associated with lower QoL as measured by both the EQ-5D VAS score and the un-weighted EQ-5D score.

0838

The effect of an education programme (HyPOS) to treat hypoglycaemia problems in insulin dependent diabetes patients

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Background and Aims: In a randomised, prospective multi-centre trial the effect of an education programme (HyPOS) for patients with hypoglycaemia problems was studied. HyPOS consists of 5 lessons. It focussed on the topics: causes and improvement of hypoglycaemia unawareness, modification of dysfunctional glycaemic targets and health beliefs

Materials and Methods: Patients with hypoglycaemia problems were eligible. 164 patients were randomised either to a control group (CG), receiving a standardised training aiming at intensive insulin therapy (n=80), or to HyPOS (n=84). Hypoglycaemia awareness was measured by the hypoglycaemia awareness questionnaire (HAQ) and by a visual analogue scale (VAS). Mild hypoglycaemic episodes (HE) were assessed by data provided by blood glucose memory meters (% of blood glucose <3.9 mmol/l).

Results: At baseline there were no differences between HyPOS and CG (age: 46.2±11.6 yrs. vs. 45.9±13.3 yrs.; diabetes duration: 20.3±10.7 yrs. vs. 22.1±10.9 yrs.; HbA1c: 7.1±0.9% vs. 7.4±1.1%; HAQ: 4.8±1.6 vs. 5.0±1.7; VAS: 4.3±2.4 vs. 4.3±2.3; HE: 17.6±9.8% vs. 16.0±9.2%; severe hypoglycaemic episodes (SHE) treated with glucose or glucagon injection: 0.93±2.2 vs. 1.07±2.1 events per patient and year). After a 6 months Follow-up the drop out rate was 10.3%. Hypoglycaemia awareness significantly improved in HyPOS compared to the CG (VAS-Δ:+1.8±2.1 vs.+1.3±2.1, $p<0.05$; HAQ-Δ: −2.7±1.9 vs. −2.4±2.1 p=.01). The number of HE was significantly reduced (p=.015) by 3.3±7.4% in HyPOS compared to 0.3±6.4% in the CG. Also the number of SHE dropped to 0.28±1.0 in HyPOS compared to 0.45±1.3 events per patient and year in the CG (p=.12).

Conclusion: HyPOS improved hypoglycaemia awareness significantly. Furthermore HyPOS reduced significantly the occurrence of HE. Hypoglycaemia unawareness and the occurrence of mild hypoglycaemic episodes play an important role in the pathogenesis of hypoglycaemia associated autonomic failure. Thus HyPOS could contribute to an improvement of hypoglycaemic associated autonomic failure. Regarding the rather short follow up period the reduction of SHE in HyPOS is also remarkable. HyPOS seems to be effective to treat hypoglycaemia unawareness.

0839

Accuracy of the new HemoCue® Monitor Glucose Measuring System assessed using patient samples and an isotope dilution mass spectrometry reference method

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Background and Aims: HemoCue® AB, Ängelholm, Sweden, has developed a new glucose measuring system, the HemoCue Monitor (HCM). This new system has been developed and is designed primarily as a glucose meter for self-monitoring of blood glucose. The instrument has an extended measuring range compared to previous HemoCue glucose systems, with an upper concentration limit of 27.8 mmol/L (500 mg/dL) for whole blood or an upper limit of 30.9 mmol/L (555 mg/dL) for the plasma equivalent version of the system. Moreover, the microcuvettes of the HCM system have a new design and are stable at room temperature. In this study the accuracy of the new system is compared to an isotope dilution mass spectrometry (ID GC-MS) method using venous patient samples.

Material and Methods: One hundred thirty six venous EDTA whole blood samples were collected over a wide concentration range, mainly from diabetics. Glucose concentrations were measured by split-sample technique using both the HCM instrument and the ID GC-MS method. To measure the between-run variation in the ID GC-MS method, controls samples at three concentration levels were analyzed together with the patient samples. Also, a control sample supplied by HemoCue was analyzed with the HCM system. The HCM microcuvette requires 4 μL of whole blood which is hemolyzed in the cuvette and the glucose is measured by means of the glucose dehydrogenase method. A chromogen in the cuvette results in an absorption proportional to the glucose concentration in the sample. For the ID GC-MS method 50 μL of whole blood was taken and pipetted into 200 μL of internal standard solution. Glucose was quantified as its aldononitrile pentaacetate on a HP MSD 5970 mass spectrometer (Clinical Chemistry 43:794–800,1997). The glucose used as calibrator in the ID GC-MS method was SRM 917b from NIST.

Results: In total 136 whole blood samples were analyzed and no results were excluded. The glucose concentration in the samples ranged from 1.6 mmol/L (29 mg/dL) to 27.1 mmol/L (488 mg/dL). Linear regression analysis comparing the HCM and the ID GC-MS results, showed a slope of 0.986 and an intercept of 0.038. The correlation coefficient was r=0.998. The mean glucose concentration of the 136 samples analyzed by the ID GC-MS method was 10.556 mmol/L (190 mg/dL), and 10.364 mmol/L (187 mg/dL) for the HCM method. Thus the bias for the HCM instrument was −0.192 mmol/L (−1.8%) compared to the reference method. No proportional error was found. The ID GC-MS method had a between-run relative variation of 0.86% ((mean concentration 4.45 mmol/L (80 mg/dL)); 2.11% (mean concentration 1.87 mmol/L (34 mg/dL)) and 1.34% (mean concentration 11.16 mmol/L (201 mg/dL)) for the control samples Seronorm, Pathonorm Low and Pathonorm High, respectively. The total variation for the HCM system measured with the HCM control sample was

1.3%. Of all the 136 glucose results from the HCM system, 89.0% (n=121) differed less than±10%, 96.3% (n=131) differed less than±15% and 98.5% (n=134) results differed less than±20% from the ID GC-MS reference method. These comparison results means that all glucose results of the HCM system compared to ID GC-MS are well in agreement of the demands from the International Standardization Organization.

Conclusion: The accuracy of the glucose analyzer HemoCue Monitor compared to the ID GC-MS reference method was very good and fulfilled the requirements for *in vitro* blood glucose monitoring systems stated of the International Standardization Organization.

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Diet and natural remedies

0840

Improvement of endothelial function in diabetic subjects after consumption of flavanol-rich cocoa

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Background and Aims: Endothelial dysfunction characterized by a reduced bioavailability of nitric oxide (NO) is a key event in arteriosclerosis, and Diabetes mellitus is associated with rapidly progressive arteriosclerosis in humans. Recently, it could be shown that endothelial function in smokers was substantially improved after ingestion of a flavanol-rich diet. The present study was aimed to investigate the effects of a cocoa drink rich in flavanols on endothelial function in diabetic subjects.
Materials and Methods: Ten diabetic patients received orally administered cocoa containing 90, 385 and 975 mg of total flavanols during a randomized, double-blind, crossover study. Endothelial function was measured before and up to 6 hours after cocoa ingestion by flow-mediated dilation (FMD) of the brachial artery using high resolution ultrasound. In parallel circulating NO-species in plasma (RNO, Nitrite, Nitrate) were characterized by reductive gas-phase chemiluminescence.
Results: Endothelial dysfunction was found in every diabetic individual under control conditions (FMD 3,71±0,27%). Ingestion of flavanols dose-dependently increased FMD to a maximum of 5,54±0,35% ($p<0,01$) after two hours. This increase was accompanied by significant augmentations in plasma concentrations of nitrite (baseline: 173±26 nM; maximum post ingestion: 293±38 nM), indicating an improvement of NO-bioavailabillity. Endothelium-independent arterial dilation was unaffected by the intervention.
Conclusion: This study for the first time provides evidence that cocoa rich in flavanols can improve endothelial function and is associated with an increase in circulating NO-species in diabetic patients. These findings may implicate a potential use of flavanols in dietary or therapeutic interventions aimed at treating Diabetes-associated vascular diseases, including peripheral and coronary artery disease that are characterized by impaired regional NO production.
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0841

Corosolic acid reduces hypertension, oxidative stress and inflammation in a rat model of metabolic syndrome

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Background and Aims: Banaba leaves (Lagerstroemia speciosa LINN.) tea is a popular drink in Southeast Asia and it has traditionally been used to treat diabetes mellitus. Corosolic acid (CRA) is one of the most pharmacologically important compounds contained in Banaba leaves. We previously found that CRA exerts its anti-diabetic effects by reducing hepatic glucose output *in vitro* and improving insulin sensitivity *in vivo*. The metabolic syndrome (MS) is characterized by a group of multiple metabolic risk factors, such as obesity, insulin resistance and elevated blood pressure. It is also known that MS is a proinflammatory state with excessive oxidative stress. In this study, we investigated the protective effects of CRA on MS using SHR/ND mcr-cp(cp/cp) (SHR-cp) rats, an animal model of MS, which develop obesity, hyperinsulinemia, hyperglycemia, hyperlipidemia and hypertension.
Materials and Methods: 6-week-old male SHR/cp rats were fed high fat diet containing 0.072% CRA for 14 weeks. We measured the plasma levels of thiobarbituric acid-reactive substances (TBARS), and 8-hydroxydeoxyguanosine levels (8-OHDG) as oxidative stress biomarkers and 3-nitorotyrosine, 3-chlortyrosine, and high-sensitive C-Reactive Protein (hsCRP) as inflammatory biomarkers, as well as body weight, blood pressure, and plasma free fatty acid.
Results: Body weight gain was slightly smaller in the CRA-treated group than in the untreated group during the

experiment, although it was not significant. Blood pressure levels at 8 weeks was significantly lower in the CRA-treated group (CRA,180±2 : Control, 200±5 mm Hg, p<0.05), which was also observed at 12 weeks (CRA,188±5 :Control, 209±3 mm Hg, p<0.05). Free fatty acid was significantly decreased in the in CRA-treated group at 2 weeks (CRA,1.0±1.2 : Control,1.3±1.5 mEq/L, p<0.05). CRA decreased oxidative stress markers as early as at 2 weeks; TBARS (CRA,2.7±0.1 :Control, 3.7±0.1 nmol/ml, p<0.05) and 8-OHDG (CRA,4.0±0.5 :Control, 9.8±0.3 pmol/ml, p<0.05), which continued throughout the experimental period. CRA also reduced inflammatory markers; 3-Nitrotyrosine (CRA,21.1±1.2:Control, 34.0±2.5 pmol/mg protein, p<0.05) and 3-Chlorotyrosine (CRA,17.4±0.6:Control, 28.3±1.3 pmol/mg protein, p<0.05) at 10 weeks, and hsCRP (CRA,222+14:Control, 268±16 μg/ml)at 12 weeks.

Conclusion: These data demonstrate that CRA ameliorates hypertension, lipid metabolism, oxidative stress and inflammation in SHR-cp rats. Together with its insulin sensitizing effect, CRA will show beneficial effects on metabolic syndrome, which has become a growing health-care problem in the world.

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0842

Natural sweetener stevioside ameliorates metabolic disturbances and attenuates hypertension in rats with fructose-induced insulin resistance

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Background and Aims: It has been shown that stevioside (S), a sweet-tasting diterpene glycoside isolated from Stevia rabaudiana Bertoni leaves, exerts antihyperglycaemic, antihypertensive effects and improves lipid profiles and oxidative status in diabetic animals. The aim of the study was to explore the impact of S on changes in metabolism and blood pressure in rats with fructose-induced insulin resistance.

Materials and Methods: Male Wistar rats were divided into three groups: the control group (C, n=8), the high fructose-fed group (F, n=8), which had free access to 250 g/L solutions of fructose for 8 weeks and fructose-fed group treated with stevioside (F+S) for 8 weeks (0.2 g/kg/day per os). At the end of the study fasted rats were subjected to the glucose tolerance test (GTT, 3 g/kg i.p.). Indexes of insulin resistance (IR) were determined using the homeostasis model assessment methods (HOMA) and quantitative insulin sensitivity check index (QUICKI). Serum levels of non-esterified fatty acids (NEFA), triglycerides (TG) and alanine aminotransferase (ALT) were measured as parameters of insulin resistance. Activity of hepatic glucose-6-phosphatase (G6P) was also measured in all experimental groups. Oxidative status was estimated by lipid peroxidation intermediates - plasma thiobarbituric acid reactive substances (TBARS) and total antioxidant activity (TAA).

Results: Fructose feeding induced insulin resistance, as indicated by higher HOMA-IR index (F: 4.71±0.70 vs C: 1.49±0.01, p<0.001) and lower QUICKI (p<0.01) indexes. The fructose group also developed glucose intolerance, hypertriglyceridemia, significantly elevated NEFA levels, ALT and G6P activities (p<0.01). Administration of S protected against the forming of fructose-induced glucose intolerance. Area under curve over the GTT was F+S: 1064±49 mM x hour vs F: 1403±90, p<0.01; C:847±25. The treatment with S decreases insulin resistance (HOMA-IR index F+S: 2.49±0.18, p<0.01), triglyceride concentration (F+S: 0.593±0.058 vs F: 0.934±0.93 mmol/l, p<0.01), NEFA levels (2.03±0.13 vs F: 4.10±0.39 mmol/l, p<0.01) and serum ALT activity (p<0.02) in compared with fructose-fed rats treated with placebo. Activity of G6P was reduced by 42% (p<0.01) in liver of fructose-fed animals after administration of S. In addition, the use of S was associated with decrease in TBARS concentration 1.5-fold (p<0.02) and increase in TAA by 25% (p<0.01) compared to control fructose-fed rats. S administration resulted in a significant decrease in mean of arterial pressure in fructose-fed rats (F+S: 123±7.0 vs F: 155± 8.0 mm Hg; P<0.05).

Conclusion: These data demonstrate that stevioside administration can ameliorate the deleterious effects of high-fructose diet such as hypertension, insulin resistance, hypertriglyceridemia, increased gluconeogenesis and oxidative stress.

0843

Efficacy and tolerability of oral stevioside in patients with type 2 diabetes: a long-term, randomized, double-blinded, placebo-controlled study

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Background and Aims: Stevioside, a natural glycoside, have in animal and human studies shown to possesses a dual beneficial action i.e. reduces blood glucose as well as blood pressure and may have a potential role in the treatment of type 2 diabetes mellitus and the metabolic syndrome. The aim of the study was to carry out a randomised, placebo-controlled, double blind, parallel, long-term trial in type 2 diabetic subjects studying the impact of stevioside on the glycaemic control, insulin sensitivity, hormone responses, lipids, weight development, and diurnal blood pressure i.e. the most important features of the metabolic syndrome.
Materials and Methods: 55 type 2 diabetic subjects aged 40–70 years were included. The subject received 500 mg stevioside or placebo (maize starch) thrice daily in a period of thee months. A twenty four hour blood pressure measurement and a meal test were carried out in the beginning and at the end of the study. The patients were monitored during the study according the protocol, re. BW, fasting blood glucose, blood pressure and lipids. At the end of the study, an IV glucose tolerance test was performed. The diabetic subjects stopped to take there p.o.antidiabetic agents two week before the onset of the study and until its end.
Results: The HbA1C was significant increased for the placebo group ($p<0.01$), as the stevioside group was unchanged ($p<0.14$) during the 3 months of treatment. In the placebo group an increase in the fasting blood glucose was detected ($p<0.007$), but not for the stevioside group ($p<0.1$). Compared to placebo, stevioside did not reduced the incremental area under the glucose response curve (IAUC) ($p<0.1$) either the meal test or the IVGTT test as well as BW. The insulin response was reduced by (45%) for the placebo group but maintained for the stevioside group. In the IVGTT test the stevioside group increased the insulin responses after injection of glucose about 21%, but it was not significant compared to the placebo group. Lipids like TG, FFA and Cholesterol did not change between the two groups, as well as the blood pressure, which may be due to the fact that the subject in both groups continue there normal hypertensive treatment during the study. **Conclusion**: We found that the group treated with stevioside did not have a decrease of insulin level in the meal test, as it was observed for the placebo group after 3 months of treatment. Insulin response was higher for the stevioside group in the GTT test compared to placebo. HbA1C and fasting blood glucose was significant increased for the placebo group, but unchanged for the stevioside group. No difference in lipids or blood pressure was observed. The patients included in this study may already have developed a late stage of diabetes, and thereby a limited beta cell function, which may explain the difference in data found in animal studies. A group of newly diagnosed type 2 diabetes patients, medicine naïve, would be preferable to confirm the anti-diabetic action of stevioside.
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0844

Differential acute effects of two different isocaloric meals, one rich in saturated fat and one rich in monounsaturated fat, on endothelial function in subjects with type 2 diabetes mellitus

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Background and Aims: Data on the effect of monounsaturated fatty acids (MUFA) on endothelial function in subjects with Type 2 Diabetes Mellitus (T2DM) are limited and controversial. The aim of this study was to examine the acute effects of two different meals, one rich in MUFA and one rich in saturated fatty acids (SAFA) on endothelial function in individuals with T2DM.
Materials and Methods: A total of 19 participants with T2DM (13 men and 6 women, mean age 58.6±10.3 years, mean duration of diabetes 4.0±3.4 years), were examined before and after consumption of two different isocaloric meals (about 560 Kcal each), one MUFA-rich and one SAFA-rich meal. The MUFA-rich meal contained 100 g toast bread and 33 g extra virgin olive oil and the SAFA-rich meal 100 g toast bread and 40 g butter. The two meals were given on two different occasions, 7–10 days apart, and in random order. Endothelial function was assessed by measurement of flow mediated dilatation (FMD) by the use of high resolution ultrasonography at baseline, 2 h, 4 h and 6 h after the test meals. In addition, plasma lipids and glucose levels were measured at the same time intervals.
Results: FMD did not change significantly over time after consumption of the MUFA-rich meal (baseline: 7.2±0.8%; 2 h: 5.0±0.9%; 4 h: 6.8±1.1%; and 6 h: 7.1±1.3%, ANOVA for repeated measurements P=0.27). A progressive significant decline, however, was observed after consumption of the SAFA-rich meal (baseline: 7.6±1.5%; 2 h: 5.5±1.3%; 4 h: 1.8±0.8%; 6 h: 3.9±1.1%, ANOVA for repeated measurements P=0.002), reaching a nadir (P value in comparison with the baseline value <0.001) at 4 h. The FMD throughout the experiment, expressed as area under the curve (AUC) after adjustment for the baseline values, increased after the MUFA-rich meal (+5.2±2.5%) and decreased after the SAFA-rich meal (−16.7±6.0%) (Δ=−11.5±6.4%, P value for the comparison of the AUCs

of the FMD change between the test meals=0.008). Plasma glucose and triglycerides concentrations increased postprandially to the same degree after both test meals.
Conclusion: In subjects with T2DM, the consumption of a SAFA-rich meal exerts a detrimental effect on endothelial function. On the contrary, a MUFA-rich meal does not result in an impairment of the endothelial function.

0845

Improvement of insulin resistance after diet with a whole grain based dietary product: results of a randomised, controlled cross-over study in obese subjects with elevated fasting blood glucose

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Background and Aims: Subjects with obesity and elevated fasting blood glucose are at high risk of developing type 2 diabetes. This risk may be reduced by a dietary intervention that leads to an improvement of insulin resistance.
Materials and Methods: We investigated the potential of a whole grain based dietary product (WG) with reduced starch content derived from double-fermented wheat (Degussa Food Ingredients GmbH) to positively influence body weight, fasting blood glucose, insulin resistance and lipids in comparison to a leading, commercially available nutrient-dense meal replacement product (MR). Thirty-six obese subjects (BMI>29 and <40 kg/m^2) with elevated fasting blood glucose (>6.1 and≤7.1 mmol/l) were enrolled in a randomized two-way cross-over study with two 4-week treatment periods separated by a 2-week wash out with habitual diet. Subjects were instructed to replace at least two meals (preferentially breakfast and dinner) with WG or MR, respectively, targeting for a daily consumption of 200 g of either product. During diet periods total daily calorie intake was limited to 1700 kcal.
Results: Thirty-one subjects (age 51±13 y, BMI 33.9± 2.7 kg/m^2, fasting blood glucose 6.4±0.3 mmol/l; mean±SD) completed the study. Both, after diet with WG and with MR body weight (−2.5±2.0 vs. −3.2±1.6 kg), fasting blood glucose (−0.4±0.5 vs. −0.5±0.8 mg/dl), total cholesterol (−0.5±0.7 vs. −0.6±0.7 mmol/l), LDL-cholesterol (−0.7±0.6 vs. −0.3±0.5 mmol/l) and triglycerides (−0.3±0.2 vs. −0.3±0.1 mmol/l) decreased significantly (ANOVA) as HOMA insulin resistance score (−0.7±1.2 vs. −1.1±2.3 μU/ml x mmol/l) improved. However, after adjustment for body weight loss (COANOVA), the improvement of fasting blood glucose and HOMA-IR score remained statistically significant only for WG but not for MR - indicating an effect of WG on fasting blood glucose and insulin resistance independent from changes in body weight.
Conclusions: We conclude that a diet with WG beneficially influences metabolic risk factors for type 2 diabetes thus potentially contributing to postpone the development of the disease.
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0846

Fiber diet ameliorates glucose homeostasis in a rat model of fructose-induced insulin resistance

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Background and Aims: The long-term administration for up to 50 days of either an olive oil- or guar-enriched diet to normal rats was recently found to improve glucose tolerance and increase the secretory response of insulin-producing cells to oral glucose. The influence of these two diets on insulin sensitivity was now explored in a rat model of fructose-induced insulin resistance.
Materials and Methods: Four groups of 8–13 male Wistar rats each were given free access to tap water and a standard diet (C) or fructose dissolved in tap water (20%, w/v) and either the standard diet (F) or a diet containing 15% (w/w) olive oil (FO) or 20% (w/w) guar (FG). After 65 days, a hyperinsulinemic-euglycemic clamp was performed over 90 min in overnight starved condition, insulin being infused at 0.4 U per hour and per g body weight.
Results: Compared to C, F displayed decreased food intake (−37%; p<0.0002), lower gain in body weight (−17%; p<0.004), increased overnight fasting plasma glucose (p<0.03) and insulin (p<0.04) concentrations, and higher (p<0.04) HOMA (insulin resistance). During the hyperinsulinemic-euglycemic clamp, the amount of infused glucose was 18% lower (p<0.0003) in F than in C. In the FO rats, the daily food intake and gain in body weight were restored to normal values; however, none of the other metabolic variables differed significantly in F and FO. When comparing FG to F, the food intake remained reduced (p<0.0001 *vs* C), and the overnight fasting plasma glucose concentration was restored to a normal level. No other significant differences in metabolic variables were found between FG and F.
Conclusions: The present results indicate that, in a rat model of fructose-induced insulin resistance, the long-term administration of an olive oil-enriched diet opposes the

lowering effect of the ketohexose on food intake and body weight gain. The long-term administration of a guar-enriched diet prevents the fructose-induced increase in glycemia otherwise recorded after overnight starvation, whilst maintaining a low food intake. These findings argue in favour of guar, rather than olive oil, intake to oppose the effect of exogenous fructose on glucose homeostasis.

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0847

Partial substitution of proteins of animal origin by soy drink in the diet of persons with type 2 diabetes. Effect on metabolic control, inflammatory markers, lipid profile and albumin excretion rate

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Background and Aims: Soybean is a high-quality protein product rich in isoflavones. Consumed at quantities around the 25 g/day of soy protein, it has been associated with several health beneficial effects, namely cardiovascular risk reduction. The aim of our study was to evaluate the effect of consuming moderate quantities of soy in the metabolic control, inflammatory markers, lipid profile and albumin excretion rate (AER) of persons with type 2 diabetes (T2D).
Materials and Methods: We studied 63 persons with T2D (35 males; 28 females) randomized by two dietetic interventions: *Group I* (n=26) with a diet according to ADA guidelines and *Group II* (n=37) following a diet with the same macronutrient composition but with partial substitution in proteins of animal origin by soy drink (2L per week=75 g of protein). Visits were made at months 0, 1, 2, 4, 6, 9 and 12 for dietetic monitoring and for the determination of HbA1c, high-sensitivity C reactive proteín (hs-CRP), erythrocyte sedimentation rate (ESR), leucocytes, fibrinogen, total cholesterol, HDL-c, LDL-c, triglycerides, apolipoprotein B (apoB), urea, creatinin and AER (night period). For each parameter, we determined the variation between basal value and that obtained in the month considered and compared delta values between the two intervention groups. Patients with inflammatory/infectious episodes were excluded as well as those with pharmacological changes or dietetic non-compliance during follow-up.
Results: Patients presented with mean age=61±9 yrs, BMI=30.5±5.9 kg/m^2, HbA1c=7.1±0.7%, hs-CRP=4.1±4.3 mg/l, ESR=15.6±13.8 mm/h, leucocytes=7.2±1.9×10^6/ml, fibrinogen=363.7±81.3 mg/dl, total cholesterol=188.7±32.6 mg/dl, HDL-c=47.8±10.2 mg/dl, LDL-c=122.4±26.1 mg/dl, triglycerides=146.4±70.7 mg/dl, apoB=90.8±15.9 mg/dl, urea=41±12.2 mg/dl, creatinin=1±0.2 mg/dl and AER=55.4±175 μg/min. At baseline AER was abnormal in 15 patients (23.8%); 13 patients (20.6%) were on insulin therapy and 32 (50.8%) on lipid lowering drugs. Globally we found a progressive increase in HbA1c throughout the study. That was significantly lower in *Group II*: month 1 (p=0.075), month 2 (p=0.023), month 6 (p=0.014), month 9 (p=0.003) and month 12 (p=0.047). A tendency for increase in triglycerides and reduction in inflammatory markers was observed in *Group II* comparatively to *Group I*: triglycerides (p=0.073 and p=0.046; respectively in month 1 and 6), ESR (p=0.008) in month 6; leucocytes (p=0.041) and fibrinogen (p=0.069) in month 12.
Conclusion: Partial substitution of proteins of animal origin by soy, for short/intermediate periods, does not induce any reliable effect in lipid profile, inflammatory markers or kidney function. However, moderate consumption of soy is associated with a consistent beneficial effect in metabolic control of persons with T2D.

0848

High fibre intake, but not low energy intake, promotes the reduction in visceral adipose tissue mass and hepatic lipids during weight loss

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Background and Aims: Weight loss reduces visceral adipose tissue mass (VAT) and improves insulin sensitivity. It is unknown whether diet composition during weight loss provides beneficial effects on body composition and ectopic fat storage in the liver beyond the effects of energy restriction alone.
Materials and Methods: 99 subjects (38 males, 61 females, age 45±11 years, BMI 29.2±4.5 kg/m2) were included in a 9 months interim analysis of the Tuebingen Lifestyle Intervention Program (TULIP). Insulin sensitivity was estimated from the oral glucose tolerance test (oGTT). In all subjects body fat depots were quantified using magnetic resonance imaging and spectroscopy. Food intake was estimated from dietary records of 15±1 representative days during the study period per subject. Nutrient intake was analyzed using a validated computer program (DGE-PC 3.0, Deutsche Gesellschaft für Ernährung).
Results: During lifestyle intervention subjects ingested 1820±30 calories and 25±6 g of total fibre per day. BMI (from 29.2±0.5 to 28.2±0.4 kg/m2, p<0.0001) and VAT

(from 3.5±0.2 to 3.1±0.2% total body weight, p<0.0001) and hepatic lipids (HL) (from 5.5±0.6 to 3.7±0.4% signal, p<0.0001) were reduced. In a multivariate regression model the relative reduction in BMI was negatively and independently associated with both, total energy (p=0.002) and the total amount of fibre (p<0.0001) ingested per day. In contrast, the reduction in VAT was associated only with the total amount of fibre (p<0.0001) ingested per day but not with ingested energy (p=0.17). The same results were obtained for hepatic lipids (p=0.02 for the effect of fibre intake). In addition, the relative reductions in BMI (p=0.01), VAT (p=0.01) and HL (p<0.01) were associated with the increase in insulin sensitivity.

Conclusion: Both, energy restriction and high fibre intake independently promote weight loss. High intake of dietary fibre results in beneficial effects on body composition and ectopic fat storage in the liver independent and beyond the effects of energy restriction alone.

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0849

Postprandial mononuclear NF-kB activation is independent of the AGE-content of a single meal

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Background and Aims: Dietary uptake of Advanced Glycation Endproducts (AGE) is supposed to potentially contribute to inflammatory reactions linked to vascular dysfunction and late diabetic complications. One mechanism by which dietary AGE might exert these effects is by activation of the proinflammatory transcription factor NF-kB. The aim of this study was to analyze the postprandial effects of a casein meal with low or high AGE content on postprandial NF-kB activation in peripheral blood mononuclear cells (pBMC) of healthy volunteers.

Materials and Methods: Casein was heated for 40 h at 50°C in the presence of sorbitol or glucose, resulting in either minimal (Sorbitol (S)-casein) or large (glucose (G)-casein) amounts of AGE-modified casein. 9 healthy volunteers ate 250 g of both types of casein, whereas both meals were separated at least by 2 weeks. Plasma and pBMC were taken before and 2 h after each meal. Thereafter, the defined AGE carboxymethyllysine (CML) was determined by ELISA and Western blot. NF-kB activation in pBMC was assayed using Electrophoretic Mobility Shift Assays (EMSA) and Western blot analysis.

Results: S-casein contained only minor amounts of CML and no pentosidine, while G-casein contained large amounts of both. 2 h after ingestion, the S-casein or the G-casein-meal, both, resulted in a non-significant increase in plasma CML and in the intracellular CML-content of pBMC. This was paralleled by a highly significant increase in postprandial mononuclear NF-kB binding activity. Remarkably, neither the extent of NF-kB induction (178% for S-casein, 188% for G-casein), nor the composition of the NF-kB heterodimer were significantly different after intake of S-casein or G-casein. Consistently, western blots confirmed an increased NF-kBp65 nuclear translocation and a decrease of NF-kBp65 in the cytoplasm, while no difference in postprandial NF-kB nuclear translocation was observed following intake of S-casein or G-casein.

Conclusion: Postprandial mononuclear NF-kB activation after a single meal is independent of the AGE-content of the ingested protein.

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PS 68

Novel therapies (preclinical)

0850

Inhibiting glycosphingolipid synthesis improves glycaemic control and insulin sensitivity in animal models of type 2 diabetes

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Background and Aims: Gangliosides are sialic acid-containing glycosphingolipids that can modulate the activity of various cell surface receptors, including the insulin receptor (IR). Previous studies have suggested that elevated ganglioside levels may lead to insulin resistance. We have developed a small molecule inhibitor of glucosylceramide synthase, the enzyme involved in the first step in the synthesis of gangliosides and other glycosphingolipids. We investigated the effects of this inhibitor (Genz-123346) on glucose homeosta-

sis and insulin sensitivity in the Zucker Diabetic Fatty (ZDF) rat and the Diet-induced Obese (DIO) mouse.

Materials and Methods: ZDF rats, 6 weeks old, were gavaged daily with Genz-123346 (75 mg/kg/day) for 6 weeks. Body weight, food consumption, glucose, glucose tolerance, hemoglobin A1c (HbA1c) and insulin levels were measured during the course of treatment. At the end of the study, various tissues including the pancreas were excised and examined histologically. For the second model, C57BL/6 mice were placed on a high fat (45% of calories) diet for 8 weeks. Obese, hyperglycemic mice were selected and gavaged daily with Genz-123346 (125 mg/kg/day) for 10 weeks.

Results: In ZDF rats, drug treatment did not have any significant effect on weight gain compared to the placebo control. Genz-123346 decreased GL1 levels in liver and GM3 levels in muscle. Genz-123346 significantly lowered glucose and HbA1c levels, improved glucose tolerance, and partially preserved pancreatic islet structure and function. In DIO mice, drug treatment normalized HbA1c levels and improved glucose tolerance. Increased activation of the IR was observed in the muscles of both the ZDF rats and DIO mice, indicating that drug treatment increased insulin signaling.

Conclusion: The glucosylceramide synthase inhibitor Genz-123346 lowered blood glucose, improved glucose tolerance, and increased insulin sensitivity in two animal models of type 2 diabetes. In ZDF rats, treatment with Genz-123346 also partially preserved pancreatic beta-cell function. The results suggest that modulating glycosphingolipid synthesis may be a novel and effective approach to treat type 2 diabetes.

0851

The effect of novel antioxidant L-2264 on glucose homeostasis and lipids peroxidation in rats with non-insulin dependent diabetes mellitus

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Background and Aims: We have previously reported that a new derivative of 1,2,4-triazolopropionamide (L-2264) decreases lipid peroxidation and corrects impairment of insulin sensitivity in rats with dexamethasone-induced insulin resistance. The aim of this study was to determine the effect of L-2264 on the glucose homeostasis and oxidative status in rats with non-insulin dependent diabetes mellitus.

Materials and Methods: Male Wistar rats (3-mo-old) were made diabetic by streptozotocin (STZ, 65 mg/kg, i.v.) given in 15 min after nicotinamide injection (230 mg/kg, i.p). One group of diabetic animals received L-2264 (100 mg/kg/day per os) for 4 weeks. Control diabetic (D) and intact groups (C) have been included in the study. At the end of the experiment rats were subjected to a glucose tolerance test (GTT, 3 g/kg i.p.) and insulin tolerance test (0.3 U/kg i.v.) as an index of insulin sensitivity (IS). Oxidative status of experimental animals was assessed by determination of malonic dialdehyde (MDA), hydroperoxides (triene, oxidiene and tetraene conjugates) contents in liver homogenate, serum total antioxidant activity (TAA) and non-etherified fatty acids (NEFA) levels in plasma.

Results: In 4 weeks after diabetes induction D rats exhibited a moderate elevation of fasting plasma glucose levels (7.4±0.3 vs C: 4.4±0.1 mmol/l, $p<0.01$), impaired glucose tolerance (area under curve (AUC) over GTT was 1502±30 vs C: 760±48 mmol/l/min, $p<0.01$) and decreased IS ($p<0.01$). L-2264-administration reduced basal hyperglycaemia (6.1±0.1 mmol/l, $p<0.05$), increased IS by 47% ($p<0.02$) and corrected impairment of glucose tolerance (AUC over GTT was 990±49 mmol/l/min, $p<0.05$) compared to D group. Diabetic rats had a greater lipid peroxidation, as indicated by the higher level of MDA (502.9±44.6 vs C: 243.6±20.6 μmol/g, $p<0.01$); triene (47.8±3.4 vs C: 27.6±1.9 mmol/g, $p<0.01$), oxidiene (17.5±2.1 vs C: 11.7±2.5 mmol/g, $p<0.01$) and tetraene (68.4±7.6 vs C:40.1±3.5 E/g, $p<0.01$) conjugates in liver homogenates and lower serum TAA ($p<0.001$) in comparison with C group. Furthermore, NEFA levels in plasma diabetic animals was increased 3-fold ($p<0.01$) compared to C. L-2264-supplementation decreased NEFA levels 2-fold ($p<0.01$) and suppressed oxidative stress in diabetic animals reducing MDA and hydroperoxides contents 1.5-2-fold compared to C ($p<0.05$). Moreover, TAA was increased (40.36±2.15% vs D: 29.85±1.56%, $p<0.01$) after administration of L-2264.

Conclusion: Treatment with L-2264 corrects glucose homeostasis and oxidative status decreasing hyperglycemia, NEFA levels, attenuating lipid peroxidation and improving free radicals defense system in rats with STZ-induced non-insulin dependent diabetes mellitus. This data justify the perspectivity of L-2264's future studies as agent for the treatment of diabetes and its complications.

0852

Combination therapy with pioglitazone and a fructose 1,6-bisphosphatase inhibitor (MB06322) improves glycaemic control and lactate homeostasis in male Zucker diabetic fatty (ZDF) rats

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Background and Aims: MB06322 (CS-917) is a prodrug of a potent inhibitor of fructose 1,6-bisphosphatase, a regulatory enzyme in gluconeogenesis (GNG), that improves glycemic control in a broad spectrum of animal models of T2DM. In male ZDF rats with advanced disease (>10 weeks of age), MB06322 treatment increases blood levels of lactate, the predominant precursor of GNG. This is not observed in younger male ZDF rats, high fat-fed female ZDF rats, or male db/db mice. Thiazolidinediones (TZDs) such as pioglitazone (PIO) are known to improve insulin sensitivity and overall metabolic state. The aim of this study was to explore the therapeutic potential of the combination of PIO and MB06322 in male ZDF rats and to evaluate potential benefits with regards to lactate homeostatasis.

Materials and Methods: Male ZDF rats (~11 weeks old) were divided into blood glucose-matched groups (n=8/group; blood glucose ~600 mg/dL) and fed Teklad 5008 chow or an admixture of this chow with MB06322 (0.2%), PIO (0.03%), or the combination of MB06322 and PIO (0.2% and 0.03%, respectively) for 3 weeks. A panel of physiological and biochemical parameters was measured at least weekly. On Day 16, satellite groups of control and PIO-treated animals were subjected to an IV lactate tolerance test (0.1 g/kg). On Day 21, leg muscle samples were harvested under anesthesia and analyzed for the expression of pyruvate dehydrogenase kinase-4 (PDK4) by RT-PCR.

Results: Combination therapy with PIO and MB06322 resulted in markedly improved glycemic control relative to the control and monotherapy groups (Table 1). Blood lactate levels increased progressively in the MB06322 monotherapy group during the first treatment week and remained ~2.5-fold elevated thereafter. Lactate elevations also occurred with combination therapy but were fully attenuated within 7 days of treatment. On Day 16, IV lactate tolerance was significantly improved in PIO-treated rats (lactate AUC 69.7±9.1 and 43.1±4.5 mM*min; controls vs. PIO). PIO monotherapy and combination therapy, but not MB06322 monotherapy, increased body weight. The expression of PDK4 in leg muscle was>60% reduced in the PIO monotherapy and combination groups relative to the control and MB06322-treated groups.

Conclusion: Two important benefits of combination therapy with a TZD and an FBPase inhibitor were noted in male ZDF rats with advanced diabetes: improved glycemic control and the attenuation of FBPase inhibitor-induced elevation of blood lactate. PDK4 is a kinase that regulates the clearance of lactate and pyruvate by inhibiting flux through the pyruvate dehydrogenase (PDH) complex. The reduced expression of PDK4 in muscle in PIO-treated rats suggests that increased flux through muscle PDH may be responsible in part for the normalization of FBPase inhibitor-induced lactacemia and improved IV lactate tolerance. The combination of FBPase inhibitors and TZDs merits clinical evaluation.

Table 1. Biochemical and physiological parameters in male ZDF rats on Day 21.

Group	Blood glucose, mg/dL	Blood lactate, mM	Body weight, g	PDK4 mRNA, relative units
Control	778±48**	2.2±0.2	393±8	1±0.1
MB06322	388±20**	5.7±0.3**	390±7	1.2±0.3
Pioglitazone	577±61**	1.9±0.2	467±13*+	0.2±0.3*+
Combination	212±17**	2.0±0.2	472±8*+	0.4±0.2*+

Data were analyzed by repeated measures or 1-way ANOVA with an appropriate post hoc test. *$p<0.05$ vs. control, +$p<0.05$ vs. MB06322, **$p<0.05$ vs. all other groups.

0853

Effects of niacin on plasma lipids of wild-type and PPAR-alpha-KO mice

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Background and Aims: In human, the effects of niacin on plasma lipids and lipoproteins share numerous features with that of fibrates: both increase HDL-C levels and plasma apoAI, reduce LDL-C levels and small dense LDL fractions and reduce VLDL-C and triglycerides. In this study, we addressed the contribution of PPARα receptors activation in the niacin actions on plasma lipids.

Materials and Methods: To assess the potential role of PPARα, wild-type C57/Bl6 (WT) and PPARα-KO mice were treated once daily for 14 days with niacin at 250, 500 or 1000 mg/kg/d, 200 or 300 mg/kg/d fenofibrate or vehicle. At the end of the treatment period, plasma total cholesterol (TC), triglycerides (TG), free-glycerol (FG) and free-fatty acids (FFA) were measured. WT animals were sacrificed and livers were collected to isolate mRNA for gene expression using Q-PCR.

Results: In three independent experiments, in WT mice, niacin provoked moderate dose-dependent reductions in TG using total glycerol determination. In the most representative experiment, in animals treated with 500 mg/kg/d of niacin, TG was 49±20 mg/dL vs 67±14 mg/dL in vehicle treated group ($p<0.05$). Concomitantly, a 10–20% decrease in FFA concentration was also observed. The lowering effects on TG resembled that obtained with 300 mg/kg/d fenofibrate (32.06±7.8 mg/dL vs 66.78±14 mg/dL, $p<0.001$). A chromatographic analysis revealed that niacin decreased FG by 50% without changing the glycerol content of the VLDL-TG

fraction. Conversely, fenofibrate decreased glycerol in the VLDL-TG fraction by 50% without modifying FG. Accordingly, fenofibrate but not niacin reduced plasma ApoCIII and ApoB concentrations (respectively 42% and 39% reduction in fenofibrate- vs vehicle-treated animals). In addition, the well-known PPARα mediated hepatomegaly in rodent was observed with fenofibrate but not with niacin. A role for PPARα in the lipid lowering action of niacin could be suspected since no effect was observed in PPARα-KO mice treated with niacin and fenofibrate. Thus we performed Q-PCR experiments on key genes involved in lipid metabolism in WT mice.

Q-PCR analysis of mRNA from liver of WT mice revealed that the expression of ACO, PDK4, UCP2, SCD1 and CD36 were not modified in the niacin treated groups. This contrasted with the large up-regulation of these PPARα target genes after fenofibrate treatments (up to 20X for PDK4). Further experiments revealed that fenofibrate reduced the hepatic expression of ApoCIII, fibrinogen beta, FAS and ACC genes in WT mice. Interestingly, such a decrease in the expression of ApoCIII, FAS and ACC could be also observed in the liver of niacin treated animals.

Conclusion: These data rule out any direct role of PPARα activation in the mechanism of action of niacin in WT mice although a cross talk with a PPARα dependent pathway cannot be excluded. Indeed, niacin as fenofibrate reduced the expression of key genes involved in hepatic triglyceride synthesis such as FAS and ACC. Whether this hepatic effect can be linked to a decrease in VLDL-TG output in dyslipidemic animals should be specifically addressed in models such as the ApoE2/E2 mice.

Supported by Merck-Sante

0854

MP-513, a highly potent and highly selective non-cyanopyrroridine DPP-IV inhibitor, achieves glucose lowering effect all day in Zucker fatty rats

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Background and Aims: Inhibition of DPP-IV is a promising approach to improve hyperglycemia in type 2 diabetes. In particular, long-lasting DPP-IV inhibitors as well as long-acting GLP-1 analogs are expected to bring the incretin effects maximally. In addition, the selectivity of DPP-IV inhibition against other peptidases, especially DPP8/9 is one of the key issues in the clinical usage. A non-cyanopyrrolidine DPP-IV inhibitor, MP-513 is currently under development for the treatment of type 2 diabetes. The aim of this study was to evaluate the enzyme inhibition profile of MP-513 *in vitro*. In addition, to clarify the beneficial effect of the long-lasting inhibition, we investigated whether once-a-day treatment of MP-513 improves glucose tolerance for a whole day in Zucker Fatty (ZF) rats.

Materials and Methods: In *in vitro* study, the inhibition property and potency of MP-513 were investigated using recombinant human DPP-IV (rhDPP-IV). The selectivity was also evaluated using crude DPP8 and DPP9. In *in vivo* study, 13 weeks old male ZF rats were subjected to two successive oral carbohydrate tolerance tests at a 12-hour interval following a single oral administration of MP-513 or vildagliptin. The concentrations of plasma glucose, insulin and DPP-IV activity were measured.

Results: MP-513 showed a competitive-type inhibition on rhDPP-IV with a Ki value of 0.41 nmol/L, and exhibited an excellent selectivity against DPP8/9 with IC50 rations of 300–700 fold. The DPP-IV inhibition of MP-513 was about 10 times more potent than that of vildagliptin, and also about 7 times more potent than sitagliptin. MP-513 at doses of 0.3 and 3 mg/kg inhibited DPP-IV activity more than 50% around 15 hours after single dosing, and clearly improved glucose tolerance accompanied with increasing insulin secretion at both 1st and 2nd carbohydrates loading in a 12-hour interval in ZF rats, i.e. the plasma glucose ΔAUC at both 1st and 2nd loading in MP-513 treatment was significantly lower than that in vehicle control. On the other hand, 3 mg/kg of vildagliptin treatment did not clearly improved plasma glucose ΔAUC in the 2nd carbohydrates loading, even though decreased in the 1st carbohydrates loading.

Conclusion: MP-513, a novel non-cyanopyrrolidine derivative showed a highly potent and highly selective inhibition on DPP-IV. Once-a-day treatment of MP-513 ameliorated hyperglycemia all day long, accompanied with long-lasting inhibition of DPP-IV and with increasing plasma insulin after carbohydrate loading. Thus, long-lasting DPP-IV inhibitor, MP-513 is expected to control plasma glucose all day long for once daily treatment, and to achieve a maximum of incretin effects in type 2 diabetes patients.

0855

The effect of vardenafil (a type 5 phosphodiesterase inhibitor) on renal function in diabetic rabbits

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Background and Aims: Diabetic nephropathy, a common cause of chronic kidney disease, can affect 20–30% of the diabetic population. The limitations of the existing treatments for diabetic renal insufficiency point to the need to develop new treatment strategies. We investigated the effect oral

treatment of the type 5 phosphodiesterase inhibitor vardenafil, has on established diabetic renal impairment in the rabbit.
Materials and Methods: Blood samples were taken from 4 and 6-month control (n=8) and alloxan-induced diabetic animals (n=8) and serum biochemical variables pertaining to renal function determined. A further 7-month sample was taken for analysis after giving control and diabetic animals either acid water vehicle (n=4 in each group) or vardenafil (3 mg/kg made up in 120 ml HCl acid water pH 4.5, n=4 in each group) to drink for 4 weeks. Spot urine samples were collected at 6 months from control and diabetic animals for the measurement of total protein and creatinine concentrations. At 7 months a 24 h-urine sample was collected to also measure the glomerular filtration rate (GFR).
Results: Renal impairment was established 4 months after the initiation of diabetes and persisted at 6 months. This was highlighted by a significant increase in serum creatinine concentration, which was significantly reduced by vardenafil treatment (6 month diabetic serum creatinine; 97+/−12.7 mmol/l; n=4 vs 7 month diabetic serum creatinine following vardenafil; 87+/−12.2 mmol/l; n=4, p=0.015), paired Student's t-test.
The total protein/creatinine ratio (TP/C) from the 6 month spot urine samples from the diabetic rabbits were significantly elevated compared to controls, indicating the presence of proteinuria, a feature of diabetic nephropathy and a predictor of renal failure. Control TP/C: n=8, 0.0116 (0.0091–0.0191) vs diabetic TP/C: n=8, 0.0227 (0.0096–0.1632), p<0.038 (Mann Whitney, unpaired test). Vardenafil treatment caused a normalisation of TP/C (n=3). Diabetic animals treated with vardenafil showed a significant improvement in GFR, when compared to diabetic animals given acid water only; GFR: diabetic acid water-treated; 6.10+/−3.68 ml/min, n=4 vs diabetic vardenafil-treated; 11.28+/−1.04 ml/min, n=4, p=0.035 (unpaired Student's t-test). Similar results were also recorded when GFR was expressed/kg.
Conclusion: One possible explanation for our results is that vardenafil induces renal vasodilatation through the formation of cyclic GMP, which improves diabetic renal function. These findings highlight a potential role for vardenafil in the treatment of diabetic nephropathy.
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0856

CS-917, a potent and selective gluconeogenesis inhibitor, was well tolerated and not associated with hypoglycaemia in normal male volunteers

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Background and Aims: CS-917 is the prodrug of a potent and specific inhibitor of the enzyme fructose 1,6-bisphosphatase, being investigated as a potential treatment for type 2 diabetes. The proposed mechanism of action is through direct suppression of abnormally elevated hepatic gluconeogenesis. Phase I clinical trials were conducted in healthy volunteers to determine the acute safety, tolerability and clinical pharmacology of this novel agent.
Materials and Methods:
Single Ascending Dose Study (SAD): Ninety-six healthy male subjects were treated with single doses of CS-917 (N=72) or placebo (N=24), administered in the morning after an overnight fast. Blinded safety data review was conducted prior to each dose escalation. In each dose panel, 8 subjects were randomized to receive double-blinded CS-917 (n=6) or placebo (n=2). Dose panels included CS-917 at 2.5, 5, 10, 25, 50, 100, 200, 400, 600, 800 and 1000 mg. Subjects were fasted for an additional four hours post-dose. Safety and tolerability were assessed and areas under the curves (AUC-0-4 hours) for glucose and lactic acid levels were calculated.
Multiple Ascending Dose Study (MAD): Thirty-three healthy male subjects were randomized in sequential escalating dose panels (6 active and 2 placebo subjects per panel) to receive once daily dosing of CS-917, administered at dosages of 100, 200, 400 and 800 mg, or placebo, for 14 days. CS-917 was administered after an overnight fast and fasting continued for an additional 6 hours. Safety and tolerability were assessed. Calculation and exploratory analysis of fasting AUC(0–6 hour) and 24-hour profiles for glucose and lactic acid were performed on Day -1 and Day+14.
Results:
SAD: No subject dropped out or was discontinued. Single doses of CS-917 were generally well tolerated. No serious adverse events occurred. Gastrointestinal adverse events were not evident at lower doses but included nausea at 400 mg, and vomiting at doses of 800 and 1000 mg. Glucose levels were not affected. No subject experienced hypoglycemia. A dose dependent effect on the area under the curve for fasting lactic acid levels was suggested at doses of 200 mg and above.
MAD: One subject withdrew consent due to personal reasons in the placebo group. No other subjects dropped out or were discontinued. Multiple doses of CS-917 were generally well tolerated. No serious adverse events occurred. Transient nausea occurred frequently in the 400 and 800 mg groups and vomiting occurred in 4/6 subjects in the 800 mg group only. Fasting glucose concentrations were not clearly affected. The area under the curve for fasting plasma lactic acid was increased in the 800 mg dose group. Mean fasting lactate levels remained within the normal range in all groups.

Conclusion: Single and multiple doses of CS-917 were safely administered to normal volunteers in the fasted state across a broad dose range from 2.5 to 1000 mg. CS-917 was well tolerated without serious adverse events or hypoglycemia. Dose limiting nausea and vomiting appeared at doses of 800 and 1000 mg. Glucose concentrations were not affected in healthy subjects. Small increases in mean lactic acid concentrations were seen only at the highest doses. It remains to be determined if potent and selective gluconeogenesis inhibitors can ameliorate the hyperglycemia of patients with type 2 diabetes. These initial safety results support further testing of CS-917.
Supported by Sankyo Pharma Development

0857

The effects of pinitol on glycaemic control and adipocytokines level in type 2 diabetes patients

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Background and Aims: Pinitol(3-O-methyl-D-chiro-inositol, DCI) is thoght to mediate the insulin action and improve glycemic control. We studied to assess the effects of pinitol on glycemic control and adipocytokines level, and the safety.
Materials and Methods: A total 66 patients with type 2 diabetes who had been taken oral hypoglycemic agents for least 3 months were enrolled in the study. They were randomly assigned to two groups and were given 400 mg pinitol or placebo three times daily for 12 weeks(Placebo, n=33, DCI, n=33). All subjects continued their current medication during the study.
Results: After pinitol therapy, HbA1c($P<0.05$), fasting glucose level($P<0.05$) and HOMA-IR($P<0.05$) decreased significantly, compared with placebo group. Such lowering effect on HbA1c was evident in subjects with poorly controlled type 2 diabetes. In the group of patietns that had HbA1c higher than 8.0% showed a greater level of reduction ($P<0.05$) than those lower than HbA1c 8.0%($P=0.16$). Also in the group of patients with HOMA-IR higher than 2.5, there was significant decrease in HbA1c($P<0.01$), compared with group of patients with HOMA-IR lower than 2.5 ($P=0.26$). There is no significant difference in change of adiponectin, leptin, free fatty acid, resistin and C-reactive protein within groups before and after treatment. No toxicity due to the pinitol was observed during the study.
Conclusion: These data support the view than pinitol can medicate the insulin action to improve glycemic control and insulin sensitivity, especially in patients with insulin resistance. There is need for further studies to elucidate the mechanism of improvement of glycemic control and insulin resistance.

0858

Insulin secretory effect of plant extracts PE080 and PE081 in perfused pancreas and BRIN-BD11 cells

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Background and Aims: PE080 (an ethanol extract) and PE081 (a choloroform extract) from the seeds of a tropical medicinal plant have previously been shown to reduce blood glucose level in Long-Evans rats. This plant is used extensively in Ayurvedic and Unani medicine for the treatment of sciatica, rheumatism and many other diseases. In the present study, the effects of these extracts were evaluated on insulin secretion together with exploration of their mechanism of action in isolated perfused pancreas and BRIN-BD11 insulin secreting cell line.
Materials and Methods: Ripe seeds of the plant were dried in an oven at 40°C and ground into a fine powder. PE080 was prepared by 80% ethanol and PE081 with n-hexane and chloroform. To study the effects of the extracts on insulin secretion Long-Evans rats (200–220 g) were anesthetized with sodium pentobarbital (50 mg/kg, ip) and the pancreas was isolated and perfused through mesenteric and celiac vessels of whole pancreas at 37°C. Both the extracts (PE080 and PE081) were dissolved at a dose of 0.1 mg/ml in Krebs-Ringer bicarbonate buffer (KRB) with BSA and dextran containing 2.8 mM D glucose. The perfusate was continuously gassed with a mixture of O_2:CO (95:50). After 20 min equilibration period the composition of the perfusate was changed every ten min with plant extracts and other reagents. Effluent samples were frozen and stored at −20°C for insulin assay by ELISA. Clonal BRIN-BD11 cells were also used to evaluate the action of PE080 and PE081 on insulin secretion. Cells were seeded in the multiwell plates at a conc of 0.15×10^6 and allowed to attach overnight. Following preincubation for 40 min at KRB supplemented with 1.1 mM glucose, cells were incubated for 20 min at 37°C with KRB, supplemented with glucose, with different concentrations of plant extracts. Aliquots were removed from each well and stored at −20°C for insulin measurement by radioimmunoassay. Cell viability was evaluated by modified neutral red assay. Results are expressed as Mean+SD for a given number of

observations(n) and compared using unpaired student t-test and Man Whitney U test where appropriate.
Results: PE080 stimulated insulin secretion in isolated perfused rat pancreas which caused a significant increase in insulin release during 10 minute perfusion with almost a 6-fold increase above basal level (Insulin, M+SD ng/ml; 0.204+0.02 basal *vs* 1.22+0.12 PE080, Peak value; p<0.001). PE081 did not evoke a significant increase on insulin secretion. The stimulatory effect of the PE080 in the perfused pancreas was inhibited by diazoxide (300 μM) (Insulin M+ SD, ng/ml; 1.22+0.12 PE080 *vs* 0.42+0.05 PE080+diazoxide; p<0.01). In BRIN-BD11 cell line PE080 stimulated insulin release in both the concentrations (50 and 100 μg/ml) compared to control (5.6 mM glucose). The higher concentration did not affect cell viability. PE081 in 100 μg/ml concentration stimulated insulin release which was also not associated with a significant reduction in cell viability.
Conclusion: These findings reveal that PE080 and PE081 exert stimulatory effects on insulin secretion mediated through a physiological insulinotropic pathway. Future work directed towards the identification of active principle (s) from PE080 and PE081 may provide interesting possibilities against diabetes.
Supported by IPICS and DAB

0859

Beneficial effects of *Momordica charantia* fruit juice on lipid and free fatty acid levels in type 2 diabetic model rats

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Background and Aims: *Momordica charantia* fruit is used as a traditional remedy for diabetes mellitus throughout the world. The hypoglycemic activity of its various extracts has been widely reported; but, its effects on lipids and, particularly on individual fatty acids, have not been adequately studied. Importance of studying the effect of plant extracts on individual fatty acids is exemplified by the report that palmitic acid has an adverse effect on pancreatic β-cell proliferation whereas palmitoleic acid has a beneficial effect. The present study was undertaken to assess the effect of *Momordica charantia* fruit juice on plasma FFA and lipid levels in type 2 diabetic model rats.
Materials and Methods: *Momordica charantia* fruit was separated from seed and crushed. The juice was squeezed out from it and freez-dried (MC-J). The rats were made type 2 diabetic by intraperitonial injection of streptozotocine to 48 h old pups and experiments were carried out 3 months after the injection. The rats were fed with MC-J at a dose of 1.25 g/kg body weight/10 ml water once a day for 28 days. The diabetic control rats were fed with deionized water only. After 28 days the rats were sacrificed and blood was collected for estimation of plasma glucose, free fatty acids and lipid profiles. Plasma glucose was measured by glucose oxides (GOD-PAP) method; triglyceride and cholesterol by enzymatic-colorimetric method and plasma free fatty acids were measured by gas chromatographic method. Significance of difference was analyzed by t-test.
Results: After 28 days fasting plasma glucose of MC-J treated rats was significantly lowered as compared to the control rats (plasma glucose in mmol/l: 9.40+0.43 in control vs 7.58+0.45 in MC-J treated rats, M+SD, p=0.001). MC-J treated rats also showed significantly lower plasma TG (p=0.001); cholesterol (p=0.04) and LDL-cholesterol (p=0.001) as compared to the control rats. Compared to control, MC-J treated rats showed a significantly lower plasma saturated FFA (% composition, M+SD, control vs treated- palmitic acid: 41.06±2.51 vs 31.01±2.17 p=0.001; stearic acid: 20.33±4.28 vs 14.67±3.92 p=0.01; arachidic acid: 4.39±1.19 vs 0.37±0.18 p=0.001 and behenic acid: 8.42±1.50 vs 1.52±0.50, p=0.001). In contrast, plasma MUFA of MC-J treated rats were significantly raised as compared to the control rats (control vs treated- palmitoleic acid: 0.89±0.11 vs 1.68±0.98 p=0.05; oleic acid: 13.32±2.29 vs 19.90±2.27 p=0.001). Plasma PUFA of MC-J treated rats were also significantly raised as compared to the control rats (control vs treated- linoleic acid: 9.49±2.03 vs 24.58±2.55 p=0.0001; linolenic acid: 0.50± 0.26 vs 1.95±01.74 p=0.0001; arachidonic acid: 0.45±0.17 vs 1.14±0.35 p=0.001). Plasma ω-3 fatty acid eicosapentaenoic acid and docosahexaenoic acid of MC-J treated rats were also significantly (p=0.001 and p=0.05 respectively) raised as compared to the control rats.
Conclusion: The data suggest that *Momordica charantia* fruit juice may have a beneficial effect through improvement of the fatty acid as well as lipid profiles in type 2 diabetes.
Supported by IPICS and DAB

PS 69

Psychological complications of diabetes

0860

Sexual dysfunction in women with diabetes

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Background and Aims: Sexual function is a neglected aspect of health in women with diabetes, though it contributes greatly towards quality of life and feeling of well being.
Materials and Methods: This study was designed to assess sexual function of married premenopausal diabetic women and ascertain the effects of glycemic control and other variates on it. 177 married premenopausal female patients of diabetes (average age 33.14±8.09 years) were administered a pre tested questionnaire, designed as a 5 point Likert scale, to assess function in the domains of sexual desire, arousal, orgasm and pain. It also assessed medical and psychological aspects of sexuality as separate domains. The control group was 100 premenopausal non-diabetic women attending the obstetric clinic in the same hospital.
Results: Women with diabetes scored highly on domain of desire (3.17±0.83). Scores on the domains of arousal and orgasm were poor (2.01±1.57 and 2.81±1.57) implying a high incidence of sexual dysfunction in women with diabetes. Scores were high for medical health related to sexuality (4.47±0.57) but comparatively low for psychological health (2.82±0.94).
15.25% of the patients experienced orgasm most or all of the time while 44.07% had never experienced it. Only 10.17% patients complained of vaginal dryness/itching or foul discharge. The incidence of painful coitus/dyspareunia was high (20.38%). 3.38% patients had history of premarital or extramarital sexual contact while 54.24% had frequent contact (>2–3 times a week). 83.05% patients reported that they would appreciate sexual counseling as a part of routine diabetes care services. Sexual dysfunction was not related to duration of diabetes, insulin therapy or glycemic control. Desire, arousal, orgasm, and pain disorders were more common in women>40 years. The self reported incidence of sensory neuropathy in this group was 33.89%.
Conclusion: This study reveals the low incidence of sexual desire dysfunction, along with a comparatively higher incidence of arousal, orgasm and pain disorders, and psychological dysfunction in premenopausal diabetic women. This underscores the need for psychological counseling to improve sexual, as well as general, health in women with diabetes.

0861

Physicians' and patients' perception of sexuality in diabetic men with erectile dysfunction. The PREDICT study

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Objectives: The aim of the survey was to provide cross-sectional data on sexual functioning in diabetic men, and to evaluate general practitioners (GP), diabetologists and patients' appraisal on erectile dysfunction (ED) and its therapy.
Design and methods: Type 1 (21%) and type 2 (79%) diabetes patients were recruited by their GPs (n=189) or diabetologists (n=24). A self-administered questionnaire designed by the study group was dispensed by an independent market research company (Medistrat N.V.).
Results: In all, 737 men with a mean age of 59 y participated to the study. Known diabetes duration was 17 years for type 1 and 8 years for type 2 diabetic subjects. Most participants (93%) had a stable relationship with their partner, lasting for>10 years in 71%. Sexuality was considered an important aspect of quality of life by 87% of participants.
About 50% of diabetic men with ED stated that their erectile capacity was severely impaired (40% at age <55 y, 44% at age 55–65 y, and 69% at age>65 y), while 10% reported minor ED. Only 31% of men with ED were previously treated with an oral PDE-V inhibitor.
In 53% of patients, medical doctors had initiated discussion on ED within a frame of potential complications arising from poorly controlled diabetes. At consultation, 21% of diabetologists and up to 55% of GP's admitted not to routinely include sexual history in regular diabetes follow-up. A more passive attitude was displayed by GP's, since they expected patients themselves to mention any sexual dysfunction. In their advice physicians stressed the importance of better metabolic control (48%), while as erectogenic agent tadalafil (67%), sildenafil (15%) and vardenafil (14%) were prescribed.
Forty-three % of patients in all age groups felt uncomfortable to discuss ED with their doctors. Forty-eight % of diabetic patients with ED considered that improving metabolic control could better or solve ED. Fast-acting PDE V inhibitors were considered to be of lesser relevance with increasing age (71% <55 y, 64% 55–64 y, 44%>65 y). Both physicians and patients considered sufficiently penile rigidity as the key component of efficacy during oral treatment of ED.
Conclusion: ED remains largely undiscussed, undiagnosed and maybe under-treated in men with diabetes, possibly

because both physicians and patients feel reluctant to discuss this problem.
Supported by Eli Lilly Belgium

0862

The role of patients' representations of foot complications in guiding adherence to foot self-care: a longitudinal study

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Background and Aims: Structural equation modelling examined the ways patients' common-sense beliefs about foot complications combine with medical information and foot ulcer experience in predicting foot self-care behaviours during an 18 month follow-up.
Materials and Methods: 495 patients with diabetic peripheral neuropathy, DN (mean age=63 y; 71% male; 72% type 2 diabetes) attending UK and USA diabetes centres (325 UK), completed self-report psychological and foot self-care behaviour questionnaires. DN was diagnosed by the Neuropathy Disability Score (NDS; mean=7.4), Patients' common-sense beliefs and interpretation of foot complications were assessed with the Neuropathy Psychosocial Inventory, NPI; foot self-care behaviour scales were based on international guidelines and covered preventive foot self-care (12 items) and potentially foot-damaging behaviours (9 items).
Results: Patients' common-sense beliefs predicted less preventive foot self-care ("good circulation=healthy feet", standardized coefficient, sc=−.10, p<0.05) and more potentially foot-damaging behaviours ("foot ulcer=pain" sc=.14, p<0.01). Accurate interpretation of medical information ("DN=nerve damage" and "foot ulcer causes") enhanced preventive foot self-care (sc=.11, p<0.05; sc=.13, p<0.01), increased perceived foot ulcer unpredictability (sc=.11, p<0.01; sc=.21, p<0.001) and anticipation of DN consequences (sc=.18, p<0.001; sc=.23, p<0.001), which, in turn, predicted preventive foot self-care (sc=.12, p<0.01). Foot ulcer history directly impacted preventive foot self-care (sc=.25, p<0.001) and potentially foot-damaging behaviours (sc=−.23, p<0.001) and indirectly enhanced preventive foot self-care by "correcting" patients' common-sense beliefs "good circulation=healthy feet" (sc=−.16, p<0.001) and "foot ulcer=pain" (sc=−.38, p<0.001). It also increased perceived ulcer unpredictability (sc=.31, p<0.001) and facilitated better understanding about foot ulcer causes (sc=.25, p<0.001). The model accounted for 30% of the variance in preventive foot self-care and 25% of the variance in potentially foot-damaging behaviours.
Conclusion: These findings highlight the importance of patients' common-sense beliefs about diabetic foot complications in shaping foot self-care and provide the basis for the development of more efficacious interventions to improve the patients' ability to manage their foot ulcer risks.
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0863

A psychometric validation of a new measure of treatment satisfaction with oral antidiabetic medication: the satisfaction with oral antidiabetic agents scale (SOADAS)

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Background and Aims: The objective of this study was to conduct a psychometric validation of the SOADAS having initially conducted patient focus groups and a comprehensive literature review.
Materials and Methods: Adults with type 2 diabetes (T2D), aged between 21 and 70, taking 1–3 OAD agents were eligible. 105 patients were recruited from sites across the US. 50% of the patients were re-tested 7 days after the initial assessment. Exploratory factor analysis (EFA) was used to assess the factor structure of the SOADAS. Reliability and validity of the SOADAS was examined. To validate the SOADAS, ancillary measures were administered to assess medication complexity and medical adherence (Morisky Medication Adherence Scale; MMAS), health status (SF-12) and treatment satisfaction (Treatment Satisfaction with Medication Questionnaire; TSQM). Clinical data were collected.
Results: The EFA presented a one-factor solution for the SOADAS. In addition, a RASCH analysis of the SOADAS was conducted to assess the uni-dimensional structure of the scale. The scale was invariant, with a non-significant chi-square (p=0.249). That is, all items were fitting the model at a Bonferroni adjusted 0.05 significance level. Internal consistency, using Cronbach's alpha, was 0.856, and indicated good internal consistency. The difference between scores at test and retest was not significant (t=1.127, df 46, p=.265). Spearman's r for total SOADAS score at initial assessment and retest was 0.711 (p<0.001), indicating test-

retest reliability. The correlation between the total SOADAS score and the global treatment satisfaction subscale of the TSQM was high (r=.707, p<0.001) indicating convergent validity. The SF-12 Physical Component Scale and the Mental Components Scale correlated moderately with the SOADAS total score (r=−0.354, p<0.001, r=−0.237, p<0.01), indicating divergent validity.

Conclusion: In summary, the SOADAS demonstrated good reliability and validity, and is ready for use to assess treatment satisfaction across a wide spectrum of OAD therapies.

Supported by GSK

0864

Determinants of psychological well-being in insulin treated persons with diabetes

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Background and Aims: Psychological well-being is an important, patient-centered endpoint of diabetes care. This study was aimed at examining psychological well-being in insulin-treated patients and determining its associations with disease-related variables and coping with diabetes as assessed by diabetes care providers.

Materials and Methods: Psychological well-being was assessed by using the WHO-5 Well-being index in 828 insulin-treated patients (49% female, aged 59±13 yrs, 13% with type 1, diabetes duration 11±8 yrs, HbA1C 8.8%±1.8, BMI 27 kg/m2±4, with neuropathy in 53%, nephropathy in 13% and retinopathy in 38% cases). The diabetes care providers were instructed to classify the patients' coping with diabetes, taking account of the prevailing emotions (positive vs negative), and approach towards information (active vs passive). Five different patterns were described as ≪Empowered and in control≫ (positive; active), ≪Fearful information seeker≫ (negative; active), ≪Tell me what to do≫ (neutral; passive), ≪Resentful victim≫ (negative; passive) or ≪Feeling fine and uninvolved≫ (positive; passive). Analysis of variance and multiple regression analysis were used to define demographic, disease- and coping-related factors affecting psychological well-being.

Results: The well-being scores in the examined subjects differed with respect to gender (male vs female 14±6 vs 13±6; p=0.01), type of diabetes (type 1 vs type 2 15±7 vs 13±6; p=0.0001), presence of neuropathy (yes vs no 11±5 vs 15±6; p<0.0001), presence of retinopathy (yes vs no 11±5 vs14±5; p<0.0001), family support (yes vs no 14±6 vs 12±6; p=0.0002), and coping as assessed by diabetes care providers. ≪Empowered and in control≫ subjects obtained the highest well-being scores (15±6) followed by ≪Tell me what to do≫ (13±6), ≪Fearful information seeker≫ (12±5), ≪Feeling fine and uninvolved≫ (11±5) and ≪Resentful victim≫ (9±5) groups. The empowered group scored better than all other groups (all p<0.01), while the ≪Fearful information seeker≫ and ≪Tell me what to do≫ groups scored better than ≪Resentful victim≫ group (p<0.0001 and p<0.0001, respectively). The subjects described as ≪Feeling fine and uninvolved≫ scored their well-being to be low. Seventy-one percent of patients from this group obtained WHO-5 scores <13, indicating possibly serious emotional problems (compared to 25% of subjects with scores <13 in the empowered group, 36% in the fearful group, 49% in the group relying on their physicians, and 74% in the ≪Resentful victim≫ group).

Multiple regression analysis indicated that psychological well-being could be predicted by gender, age, type and duration of diabetes, neuropathy, retinopathy, HbA1C, family support, and coping-related characteristics as assessed by health care providers (R2=0.195 F=13.93; p=0.0001). Being younger, free of complications, supported by family, with better glycaemic control, and approaching diabetes positively/actively independently predicted better psychological well-being.

Conclusion: Psychological well-being was found to be associated with age, gender, family support, glycaemic control, diabetes complications and coping with the disease. The self-assessed well-being seemed to be interrelated with the providers' assessments. However, poor well-being remained unrecognized in patients labeled as ≪Feeling fine and uninvolved≫. Self-assessments of psychological well-being should be incorporated into routine diabetes care.

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0865

The association between stages of change and initiation of insulin treatment in Japanese patients with type 2 diabetes

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Background and Aims: The Diabetes Attitudes Wishes and Needs (DAWN) study was designed to give a global perspective on patient and provider perceptions of diabetes management, and to identify opportunities to improve psychosocial aspects of diabetes care. Results suggest that psychological hindrance to starting insulin treatment exists in patients and healthcare professionals. In response to the DAWN study, we initiated a survey to identify psychological factors related to insulin treatment in Japan. The prospective association between stages of change in patient attitudes towards insulin and success of insulin initiation after patients were advised to start insulin was also assessed.
Materials and Methods: Patients with type 2 diabetes (n=117) reported their attitudes towards starting insulin treatment using a self-administered questionnaire at the time of receiving a recommendation to initiate insulin. The questions evaluated the patients' attitudes towards change. Agreement with each question indicated whether they had a 'preparation', 'contemplation' or 'precontemplation' attitude. The percentage of patients who had commenced insulin was assessed twice, 1 month after recommendation and at 6 months.
Results: At the time of insulin recommendation, 37 patients (31%) responded "I am thinking of taking insulin immediately" (preparation stage), 47 (40%) responded "I am wondering whether to take insulin" (contemplation stage) and 33 (28%) responded "I have no intention of taking insulin" (precontemplation stage).
At 1 and 6 months, there was a significant difference in the number of patients receiving insulin dependent on their response, both $p<0.001$ (Table). At 6 months, insulin initiation had been successful in almost all of the patients in the preparation stage.

Table. Patients using insulin 1 and 6 months after recommendation

Patient attitude at time of insulin recommendation	Percentage of patients using insulin: 1 month after recommendation	6 months after recommendation
"I am thinking of taking insulin immediately" (n=37) **Preparation stage**	87%	97%
"I am wondering whether to take insulin" (n=47) **Contemplation stage**	26%	38%
"I have no intention of taking insulin" (n=33) **Precontemplation stage**	9%	30%

Conclusions: The degree of patient willingness to start insulin at the time of their healthcare professional's recommendation directly influenced the success rate of insulin initiation. Furthermore, patient willingness to start insulin treatment increased with time, following recommendation from their doctor. When insulin is recommended, it could be useful to assess patient willingness to initiate treatment, so that personalised interventions to change patients' attitudes towards insulin may be implemented if needed.
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0866

Japanese physicians' attitudes towards insulin therapy may influence prescribing behaviour

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Background and Aims: The worldwide Diabetes Attitudes, Wishes and Needs (DAWN) study investigated psychosocial and behavioural barriers to insulin treatment experienced by patients and healthcare professionals. Nearly 50% of doctors did not begin insulin treatment until it was absolutely necessary and suggested that readiness to prescribe insulin is related to doctors' diabetes experience. In response to the DAWN study, the relationship between physicians' attitudes towards prescribing insulin and their prescribing behaviour was investigated in Japan.
Materials and Methods: Japanese physicians (n=132) participated: 75 Japan Diabetes Society (JDS)-certified specialists (treated the most patients with diabetes); 30 JDS-affiliated specialists; and 27 doctors not affiliated with JDS (treated the least patients with diabetes). JDS-certified specialists had more patients receiving insulin (34%) than JDS-affiliated specialists (23%) and non-JDS doctors (10%). Attitudes towards prescribing insulin were evaluated with a questionnaire (27 attitude statements). High scores indicate agreement with attitude statements. The association between doctors' attitudes and recommendation for insulin was assessed by multiple regression analysis.
Results: Scores for 4 attitude statements correlated with physicians' level of JDS affiliation. Non-JDS-affiliated doctors obtained the highest scores for all of the statements ($p<0.001$), Table. JDS-certified specialists had the lowest scores for three of the statements, positively correlating with level of affiliation. However, scores for the statement "It is

troublesome for me to explain about handling injectors" were negatively correlated with level of JDS affiliation.

Table. Attitude scores for different physician groups

Attitude statements	β (multiple regression analysis)	*p*	Scores JDS-certified specialists	JDS-affiliated specialists	Non-JDS doctors
"I am not familiar with insulin therapy"	0.34	$p<0.001$	1.20	1.90	2.93
"There are no in-house support staff"	0.45	$p<0.001$	1.35	2.03	3.26
"If insulin therapy is necessary the patient has to be referred to a specialist"	0.24	$p<0.01$	1.39	1.97	3.33
"It is troublesome for me to explain about handling injectors"	−0.33	$p<0.001$	1.97	1.90	3.07

Doctors recommended insulin for only 23% of patients who were defined as candidates for insulin therapy ($HbA_{1c}\geq 8\%$, receiving high-dose sulphonylureas).

Conclusions: Treating fewer diabetes patients and insulin therapy-related psychological factors may affect doctors' attitudes towards insulin treatment, delaying prescribing for patients who would benefit. Conversely, treating the most patients may also result in attitudes which delay insulin treatment. Addressing these attitudes and behaviours may enable more patients to receive timely recommendations to initiate insulin.

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0867

Treatment satisfaction in type 2 patients: Evaluating the impact of clinical outcomes, patient and disease characteristics

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Background and Aims: Treatment satisfaction (TS) differs between therapy options and impacts patient compliance, self-management behaviors and costs. Clinical wisdom assumes that TS with insulin therapy improves with fewer injections and side effects. However, little is known about the combined impact of treatment outcomes and patient characteristics on TS. This study examined the relationships between TS, patient characteristics (age, gender, ethnicity, weight, co-morbidities, diabetes duration) and treatment outcomes (HbA_{1c}, number of daily injections, weight gain, hypoglycemic events).

Material and Methods: Baseline and 28 week end of study data from a 25 site (US) RCT comparing efficacy and safety of BID biphasic insulin aspart 70/30 (BIAsp 70/30) vs. QD glargine in insulin naïve type 2 patients failing oral medication (n=240) were analyzed. Subjects completed the validated Insulin Treatment Satisfaction Questionnaire (ITSQ), assessing overall TS and 5 domains (Lifestyle Flexibility, Hypoglycemic Control, Glycemic Control, Device Satisfaction, Inconvenience of Regimen). Independent regression analyses examined the relationship between TS, baseline patient/disease characteristic and treatment outcomes. All significant factors were then examined together by multivariate regression analyses.

Results: A major predictor of overall TS, Lifestyle Flexibility, Hypoglycemic and Glycemic Control, was the presence of diabetes-related co-morbidities, notably neuropathy ($p<0.05$). Retinopathy significantly impacted Device Satisfaction, and age was related to Lifestyle Flexibility ($p<0.05$). No other baseline patient/disease characteristic impacted TS. Reduction of HbA_{1c} significantly impacted overall TS, Device Satisfaction, Glycemic Control, and Inconvenience of Regimen ($p<0.05$). Number of hypoglycemic events impacted Hypoglycemic Control satisfaction, whereas the timing of the event (day vs. night) was related to overall TS and all domains ($p<0.001$, negative impact for daytime events). Weight gain, number of daily injections and treatment group did not impact TS despite the presence of significant treatment group differences (BIAsp 70/30 significantly reduced HbA_{1c}, with increased minor hypoglycemia and weight gain). When all significant factors were examined together, HbA_{1c} reduction and neuropathy maintained their previously identified significant impacts on TS. The number of hypoglycemic events remained significant only for satisfaction with Hypoglycemic Control, and timing of the hypoglycemic event significantly impacted overall TS and 3/5 domains ($p<0.01$).

Conclusion: Contrary to clinical wisdom, the number of daily injections and the occurrence of weight gain or minor hypoglycemia may not negatively impact TS when balanced by significant HbA_{1c} treatment benefits. Further, reduced HbA_{1c} and timing of hypoglycemia, rather than number of events, may have more broad impact on TS. Factors impacting TS when examined independently did not always exert the same significant influence when

assessed in combination with other factors. Thus, clinicians should judge the balance between positive and negative treatment outcomes before considering treatment options.
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0868

Newly diagnosed patients with type 2 diabetes mellitus are satisfied with insulin treatment

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Background and Aims: Insulin treatment in type 2 diabetes mellitus (T2DM) is usually deferred until oral hypoglycemic agents fail because physicians fear side effects (mainly hypoglycemia and weight gain) and believe that patients would not accept an injectable therapy. Thus, as glycemic control deteriorates, treatment is intensified in a stepwise manner resulting in a longer exposure to hyperglycemia which increases the risk of complications and exposes the beta-cells to the potential damaging effects of hyperglycemia. Early insulin treatment could prevent progressive glycemic deterioration and might preserve the beta-cell function. The study evaluates the feasibility, acceptance, and effectiveness of insulin in combination with metformin in newly-diagnosed, treatment-naive, patients with T2DM.
Materials and Methods: Eligible patients were started on biphasic insulin aspart 30/70 (30% soluble and 70% protaminated insuln aspart) delivered by FlexPen® and metformin 1000 mg twice daily. Biochemical evaluation was done at baseline and end of the 3-month study period. Patients were seen monthly for evaluation of side effects, compliance, and insulin dose adjustments, which were based on BG logs and historical information. Patient quality of life and acceptance of insulin treatment was evaluated with the Diabetes Quality of Life Questionnaire (DQoL) at the end of the study.
Results: Of 63 patients enrolled (age 44.7, BMI 35.4, 43 males) 8% dropped out for reasons unrelated to the study intervention. HbA1c decreased from 10.8 to 5.9%, with 100% of subjects achieving HbA1c≤7%, and 90% a HbA1c≤6.5%. The weight increased from 100 to 101.5 kg ($p=0.006$), but if we account for an average of 7.2 kg lost in the 6-month preceding the diagnosis of diabetes, the net weight change was −5.55 kg ($p<0.001$), so not all the weight lost prior to diagnosis was regained. The rate of hypoglycemia was 1.5 episodes/patient-month, with a liberal definition of hypoglycemia (BG <3,9 mmol/l with symptoms). Only one severe hypoglycemic event occured. At the end of the study 84.5% of the patients rated their general health good or above and 88% reported improvement in their general health compared to baseline. 93% of the patients were satisfied with their insulin treatment, and 88% were willing to continue insulin. 81% reported they felt their diabetes was well controlled on the current treatment. Mean score for the following subscales of the DQoL was: treatment satisfaction 1.83, disease impact 1.81, impact of hypoglycemia 1.75, lifestyle flexibility 2.15, and social impact 1.18. Compliance rate with insulin was 96.7% and metformin 95.2%.
Conclusion: Outpatient initiation of insulin in combination with metformin at the time of diagnosis of T2DM is an effective, safe, and feasible strategy to rapidly achieve normoglycemia. All patients got excellent glycemic control within 3 months, had a low rate of hypoglycemia with minimal weight gain. Insulin therapy was very well accepted by the patients, refuting the misconception of low satisfaction and compliance with such treatment.
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0869

Physician beliefs in efficacy of insulin glargine (GLAR) are associated with glucose control in type 2 diabetes (T2DM)

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Background and Aims: Goal A1C (*G*lycemic *O*ptimization with *A*lgorithm and *L*abs *A*t Po*I*nt of *C*are) was a randomized, parallel group, four-arm, open-label, 24-week study to assess the impact of active (weekly monitored) vs usual monitoring of algorithmic insulin titration and point-of-care (POC) vs laboratory A1C in patients with T2DM (N=7893; mean age 57 y, weight 98 kg, diabetes duration 8.4 y, fasting blood glucose 211 mg/dL, A1C 8.9%) who failed oral antidiabetic therapy and started GLAR treatment. The objective of the present analysis was to determine if physicians' knowledge of, attitudes toward, and expectations of GLAR and its effects might be associated with glycemic control.
Material and Methods: At baseline, physicians (N=2871) were surveyed about their demographics, GLAR knowledge, reasons for prescribing GLAR, and expected efficacy and safety of GLAR. Physicians were asked to predict the percentage of patients they expected to achieve A1C <7.0%, and the percent decrease in mean A1C their patients would achieve by week 12 and week 24. They were also asked to rate their level of agreement with factors that may affect success of treatment with GLAR (1-strongly disagree to 5-strongly agree), and to rank 4 reasons for prescribing

GLAR from highest (1) to lowest (4). Reasons to be ranked were "improves blood sugar control", "flexibility of use", "fewer hypoglycemic episodes", and "positive feedback from patients".
Results: Of physicians surveyed 14% were women; 11% were endocrinologists; 79% were primary care/internal medicine and 10% were of other specialties; and 33% practiced in urban, 44% in suburban, and 23% in rural areas. Average patient A1C over the study did not differ between physician subspecialties. When patient A1C was evaluated against physician responses to the survey at baseline, we found that lower average A1C was associated with higher physician expectation in goal achievement (<7%) by week 12 ($P=0.005$). Patients whose physicians gave lower rankings to "fewer hypoglycemic events" and "positive feedback from patients" as reasons for prescribing GLAR achieved lower (better) A1C averaging over the study period ($P=0.02$ and 0.04, respectively). Physician practices that reported seeing more than 10 patients per week were also associated with better mean patient A1C ($P=0.007$). Regardless of A1C treatment group, physicians who "strongly" or "somewhat agreed" that the ease of use of GLAR will make patients more receptive to its use was associated with a higher mean patient A1C ($P=0.02$). As previously reported, more patients in POC A1C active titration group achieved A1C <7% (41% for POC vs 36% for laboratory testing; P<0.0001). Results of the current analysis showed further A1C differentiation between groups. In the POC A1C active titration group, patients of physicians who ranked "GLAR improves blood sugar control" as the most important reason for prescribing it achieved lower mean A1Cs than those who gave lower rankings to this reason ($P=0.047$). The opposite was true for laboratory testing conditions.
Conclusion: This study presents novel evidence that physician beliefs in efficacy of GLAR are an important determinant of actual glucose control in patients with type 2 diabetes treated with GLAR.
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PS 70

Psychiatric complications of diabetes

0870

Depression and treatment choice in type 2 diabetes patient failing oral antidiabetic treatment

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Background and Aims: The aim of this cross-sectional study was to assess the interaction between depression and treatment preferences regarding outcome parameters in type 2 diabetes patients failing oral antidiabetic treatment (OAD).
Materials and Methods: Patients with type 2 diabetes (N=532) on OADs (Male 66.9%) with a mean age of 56,5 yrs (sd 8,8) and a mean $HbA1_c$ of 8.34% (sd 1.5) were recruited in study sites across Germany. Participants discussed treatment optimization options with a physician and were asked to make a theoretical choice about future diabetes therapy. In Step One options were to continue with OADs or to move on to subcutaneous (SC) insulin. In Step Two an additional treatment option with inhaled insulin (INH) was offered. Questionnaires were used to assess depressive disorders (PHQ), diabetes-specific stress (PAID, higher values indicates more stress), and health related quality of life (HRQoL; SF-36, z-values).
Results: Taken both steps together, 16.0% opted for SC, 27.4% opted for INH, and 56.6% for OAD. For the whole sample a 10.0% prevalence of depression was observed. Patients reported moderate diabetes related stress (mean 23.51; sd 17.23) and HRQoL were clearly within the normal range of the general German population for the physical (mean −0.3; sd 1,0) and mental (mean −0.3; sd 1,1) component. Significant main effects for depression were observed in analyses of variances for physical ($F=87.64$; $df=1$; $p<0.001$) and mental HRQoL($F=137.00$; $df=1$; $p<0.001$). There were two significant interactions between treatment choice and depression. First, HbA1c was higher in patient who opted for SC insulin, but only for depressed patients ($F=3.91$; $df=2$; $p<0.05$). Second, diabetes-specific stress was higher in patients with depression but only for those who opted for OAD or INH ($F=6.04$; $df=2$; $p<0.01$).
Conclusion: A view at the whole sample gives the impression of relatively low diabetes related stress and normal HRQoL despite the poor metabolic control of the patients. In contrast, the subgroup of patients with depression reports strongly impaired HRQoL. The consideration of depression in interaction with the treatment choice revealed that patients with depression and extremely poor metabolic control were more likely to choose SC insulin. Participants who preferred OADs or inhaled insulin described themselves as strongly impaired by diabetes-specific stress when depression was present whereas those who opted for SC insulin seems to have very low perceived burdens of their diabetes even in the presence of depression.
Supported by Pfizer Pharma GmbH

Interaction of treatment choice, depression and diabetes outcome (mean, SD)

Treatment Choice	Depression PHQ	$HbA1_c$ %	PAID (0–100)	Physical HRQoL (z-values)	Mental HRQoL (z-values)
OAD	No Depression N=276	8.1±1.4	20.8±15.2	−0.0±0.8	−0.1±0.9
	Depression N=25	8.6±1.5	41.6±26.4	−1.6±0.7	−2.2±0.8
	Sum N=301	*8.2±1.4*	*22.5±17.3*	*−0.17±0.9*	*−0.3±1.0*
SC insulin	No Depression N=76	8.5±1.6	22.3±15.5	−0.4±1.0	−0.3±1.2
	Depression N=9	10.4±1.9	20.4±14.2	−1.5±1.0	−1.5±1.0
	Sum N=85	*8.7±1.7*	*22.1±15.3*	*−0.5±1.1*	*−0.4±1.2*
Inhaled Insulin	No Depression N=127	8.5±1.4	23.9±16.1	−0.2±0.9	−0.1±0.9
	Depression N=19	8.7±1.5	42.8±20.3	−1.4±1.0	−2.0±1.0
	Sum N=146	*8.5±1.4*	*23.5±17.2*	*−0.3±1.0*	*−0.4±1.1*
Sum Depression	No Depression N=479	*8.3±1.5*	*21.9±15.5*	*−0.1±0.9*	*−0.1±0.9*
	Depression N=53	*8.9±1.7*	*38.4±23.7*	*−1.5±0.9*	*−2.0±1.1*

0871

Depression in type 2 diabetes in primary care

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Background and Aims: Diabetes mellitus is a chronic health problem that might also influence the affective status of patients. Besides its effects on lifestyle, it has important implications for a patient's well-being and social life. The aim of this study was to evaluate the level of depressive condition in a primary care population and to investigate any relationship with socio-demographic and health outcome measure.

Materials and Methods: The intervention method was a prospective, cross-sectional survey, which was performed randomly during clinical open access encounter.The family medicine centre (FMC) is located in Bursa/West Turkey.

The sample size of the study population for type 2 diabetes was calculated as 263 (confidence level 95%, confidence interval 5+/−1%). Three hundred diabetes patients (32–87 years; 69.7% female, 30.3% male; 75.4% married and 24.6% widowed) filled out the Turkish Beck Depression Inventory (BDI) and the Turkish SF-36 health outcome measure. Information on age, sex, marital status, social security, duration of diabetes, other health problems, type of management, physical activity, perception on their quality of life and perception on their diabetes specific quality of life have been gathered with a questionnaire. Chi-Square, Student t test and logistic regression methods have been applied.

Results: Participants had type 2 diabetes since 1–37 years (mean 8.2±7.2 years). The internal validity of BDI and SF-36 revealed medium-high results (Cronbach alpha 0.70–0.89). BDI scores in 24 (8%) participants had scores of 17 and above (cut-off value for depression disorder). Female (20; 83%) suffered more than male of depression [x2 (300,1)=4,2; p=0.04]. Marital and occupational status showed significant differences [x2(300,1)=11,4 and 4,2; p<0.05]. Physically active participants had lower BDI scores [t(300,298)=2,5; p=0.01]. Patients having higher BDI scores were suffering of lower health outcome measure dimension scores (i.e. physical functioning, physical role, pain, general health, vitality, social functioning, mental health) [t(300,298)=2.3–5.5; p<0.05]. The age, perception of general quality of life, perception of diabetes specific quality of life, physical functioning sub-dimension and general health sub-dimension of SF-36 were explaining the higher BDI scores (>16) according to the logistic regression model (inclusion method).

Conclusion: Depression was prevalent in 8% of type 2 diabetics and women were suffering more in primary care setting. The age, the perception of quality of life and diabetes specific quality are predictors of depression in type 2 diabetics. It seems advisable that depressive disorders should be considered during primary diabetes care in special risk groups (i.e. women, single-divorced, elderly, perception of low quality of life).

0872

Differential effects of neuropathic symptoms on anxiety and depression: a longitudinal study

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Background and Aims: Structural equation modelling was used to examine longitudinally the role of neuropathic symptoms and foot ulceration in generating anxiety and depression.
Materials and Methods: 338 patients (mean age=63 y; 71% male) with diabetic peripheral neuropathy (DN) completed the Hospital Anxiety and Depression Scale (HADS). DN was diagnosed by the Neuropathy Disability Score (NDS; mean=7.4). Neuropathic symptoms (pain, unsteadiness and reduced feeling in feet) were assessed with a neuropathy-specific quality of life scale, NeuroQoL. Measures were completed at baseline, 9 and 18 months. To account for confounding between anxiety (HADS/A) and depression (HADS/D), and to assess factors associated with change in HADS over time, both baseline HADS/A and HADS/D scores were included as predictors of 18-month HADS/A and HADS/D.
Results: Controlling for demographic and disease variables, baseline NDS predicted 18-month HADS/A (standardized coefficient, sc=.13, p=.011) and HADS/D (sc=.15, p<0.001). Preliminary models showed that neither baseline nor changes in symptoms of reduced feeling from baseline to 9 months predicted 18-month HADS/A or HADS/D. Likewise, neither foot ulceration at baseline nor new foot ulcers during follow-up predicted 18-month HADS scores. Thus, only symptoms of pain and unsteadiness were included in the final model. The final model fitted the data well [$\times^2$ (29)=36.64, p=.16, CFI=.99, RMSEA=.02, SRMR=.03] and accounted for approximately 58% of the variance in 18-month HADS/A and HADS/D. Baseline and change in unsteadiness from baseline to 9 months predicted both 18-month HADS/A (baseline: sc=.14, p<0.001; change: sc=.09, p<0.05) and HADS/D (baseline: sc=.20, p<0.001, change: sc=.14, p<0.001). Change in pain also was predictive of both 18-month HADS/A (sc=.08, p<0.05) and HADS/D (sc=.12 p<0.001). However, baseline level of pain was predictive only of 18-month HADS/A (sc=.09, p<0.05).
Conclusion: DN predicts depression and anxiety through symptoms of pain and unsteadiness. Depression and anxiety are associated with changes in both pain and unsteadiness. However, depression is not associated with baseline levels of pain while anxiety is. This suggests that depression accommodates to pain over time, while anxiety does not. Clinicians should monitor levels of both anxiety and depression in patients who have experienced recent increases in pain and unsteadiness, as well as persistent unsteadiness. Anxiety should be monitored in patients with painful DN even in the absence of recent increases in pain.
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0873

Depressive disorders, diabetic foot ulcers and death: results from a population based cohort of people with diabetes and their first foot ulcer

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Background and Aims: The outcome for people with diabetic foot ulcers is very poor despite intensive medical regimens. The role of psychological factors in contributing to poor outcomes has not been prospectively studied. Our aim was to test whether depressive disorders increased the risk of death and morbidity in people with diabetes following the onset of their first foot ulcer over an 18 month period.
Materials and Methods: We used a prospective cohort design. We recruited a population-based cohort of people presenting with their first diabetic foot ulcer from all the community podiatry and hospital diabetic foot clinics within 5 health authorities in South London, United Kingdom representing a population of 6 million between August 2000 to October 2002. The baseline diabetic foot ulcer was defined as: i) the first ulceration in the anatomical foot (the largest ulcer if several ulcers) and ii) a full thickness break in the epithelium with a minimum width of 5 mm and iii) ankle:brachial ratio≥0.5 at either the dorsalis pedis or anterior tibial sites using Doppler pressure readings. We used the World Health Organization's Schedule for Clinical Assessment in Neuropsychiatry 2.1 face to face interview to generate Diagnostic and Statistical Manual for Mental Disorders-IV (DSM-IV) major depressive disorder at baseline. We measured the following outcomes: number of deaths of first amputation, of first recurrence and whether the ulcer had heal and time taken for each of these events to occur, censoring the event time at the time a person died or otherwise at 18 months. We measured at baseline age, sex, marital status, socioeconomic status, smoking, glycosylated haemoglobin (A_{1c}) and Texas severity of ulcer as potential confounders.
Results: 262 people presented with their first foot ulcer; 260 met the study criteria and of these 253 consented. The majority of the cohort were male, had an average age of 62 years, from social classes III-V and had type 2 diabetes. The average duration (years) of diabetes was 30.1 (SD 15.4) and 11.4 (SD 10.1) in people with type 1 and type 2 diabetes respectively. One sixth of the study population was current

smokers. The prevalence of DSM-IV major depression was 25% (n=60). 10 people were receiving an antidepressant. There were 41 (16.2%) deaths (all from natural causes), 36 (15.5%) first amputations, and 99 (43.2%) first recurrences. In the Cox regression analysis at time 18 months, DSM-IV major depression was associated with around a two-fold increased risk of mortality at 18 months (adjusted hazards ratio 2.1, 95% confidence interval 1.1 to 4.1), amputation (adjusted hazards ratio 1.7, 95% confidence interval 0.9 to 3.5) but not with healing (adjusted hazard ratio 1.3, 95% confidence interval 0.9 to 1.9) or recurrence (adjusted hazards ratio 1.2, 95% confidence interval 0.7 to 1.9).

Conclusion: Depressive disorders are very common in people who develop their first diabetic foot ulcer and is prospectively associated with an increased risk of mortality after biological confounders such as severity of ulcer are accounted for. The mediating mechanisms are as yet unclear. Depression is a reversible condition and its identification and treatment could improve the life expectancy and morbidity of people with diabetic foot disease.

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0874

What is the evidence for an increased risk of diabetes mellitus in people with schizophrenia who are prescribed atypical antipsychotics compared to those prescribed typical antipsychotics?

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Background and Aims: There is increasing concern that atypical antipsychotics may have an adverse effect on increasing the risk of developing diabetes mellitus in people with schizophrenia perhaps by inducing weight gain. There are numerous alternative explanations such age, ethnicity, obesity, polypharmacy and definition and type of psychiatric disorder, as well as selection bias in screening for diabetes which have not been consistently accounted for in the literature. Our aim was to conduct a systematic review and meta-analysis of the pooled relative risk of diabetes in people with schizophrenia who were prescribed atypical antipsychotics compared to those prescribed typical antipsychotics.

Materials and Methods: We used the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines on reporting of descriptive studies. Studies eligible for inclusion were case series, cross-sectional, case-control studies, cohort or controlled trial designs of children and adults with schizophrenia or related illness. The main outcome was diabetes mellitus. Using established Cochrane Collaboration search strategies and definitions for schizophrenia, antipsychotics and diabetes mellitus, we searched MEDLINE, PsychINFO, EMBASE, International pharmaceutical abstracts, CINAHL, and Web of knowledge from their inception to 31st July 2005 for studies in all languages. Studies were excluded if data could not be stratified into schizophrenia and related disorders versus other psychiatric disorders. Data extracted included the number of subjects with and without diabetes, type of antipsychotic medication, age, ethnicity, body mass index, and diagnostic classification used (if any) for schizophrenia and related disorders and for diabetes. The main outcome was diabetes mellitus. Corresponding authors were contacted for additional information and non-English papers were translated. The quality of the studies was assessed using the MOOSE checklist.

Results: Our electronic search found 1546 abstracts of which 230 articles were identified for full data extraction. Of these, 83 were cross-sectional, cohort or controlled studies, 69 case reports/case series and 78 review papers. To date 7 studies fulfil the criteria for inclusion in the systematic review; 4 are cross-sectional and 3 are cohort studies.

In our preliminary analysis, in the first 4 studies we identified, the unadjusted pooled relative risk of a diagnosis of diabetes in people with schizophrenia prescribed an atypical antipsychotic was 1.41 (95% confirdence interval 0.92 to 2.16) compared to being prescribed a typical antipsychotic. Further analyses of all identified studies will be presented.

Conclusion: There is a lack of high quality epidemiological studies and at present there is insufficient evidence for an increased risk of diabetes in people with schizophrenia who are prescribed atypical antipsychotics compared to those on typical antipsychotics.

0875

Alexithymia in type 1 diabetes and coeliac disease

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Background and Aims: Type 1 diabetes (T1D) and coeliac disease (CD) are chronic conditions characterized by a careful attention to diet with consequent implications on

psychological and social life. The strength to maintain a correct way of eating for lifetime is a relevant stress factor leading to psychosomatic or psychiatric disorders such as depression anxiety. In subjects with T1D the quality of life is influenced by several individual factors like personal attitude and psychopathological traits such as alexithymia, a psychological construct linked to failure in psychological self-regulation and deficit in experiencing emotional life.
It has been shown that a relationship exists between the alexithymic construct and perceived stress so that alexithymic features may interfere with the affected subject's disease-coping strategies because of a higher perceived stress. We previously reported that in T1D alexithemia is frequent, however it is not clear whether this is a specific feature of T1D or consequence of a chronic disease at onset in childhood irrespective of the type. In the present study we tested the differences in the emotive and cognitive function between subjects affected by T1D and CD through the evaluation of the alexithymic construct.
Materials and Methods: We studied 50 consecutive subjects affected by T1D (mean age 29 years, 17 males and 23 females, mean disease duration 10.8 years) and 31 subjects with CD (14 males and 17 females, mean age 31 years, mean disease duration 6.7 years) attending out patient clinics in two different hospitals. Alexithymia was assessed using a validated translated version of TAS-20 (Bagby 1994), a self-report measure of the alexithymia construct. Alexithymia was used as a continuous variable. Perceived stress was assessed by Perceived Stress Scale (PSS), a 14-item self report tool used to provide a global measure of perceived stress in daily life.
Results: According to standard evaluation parameters (alexithymia score>51), alexithymia was observed in 48% of T1D and 45.3 of CD subjects (p=NS). A positive correlation was also found between TAS and PSS (linear regression r=0.69, p<0.0001) in TD1 but not in CD. Evaluation of the three different subscales of the alexithymic construct (1=difficulty identifying and describing feelings; 2=difficulty distinguishing between feelings and body sensations; 3=constricted imaginal processes) in subjects with high and low levels of alexithymia showed a different prevalence of the three relative specific constructs both in TD1 and in CD patients.
Conclusion: This is the first study investigating whether alexithymia is a specific feature of T1D. We demonstrated that a clear relationship occurs between the alexithymic construct and perceived stress in T1D but not in CD affected subjects suggesting that disease occurrence and duration irrespective of the type of the disease cause alexithymia, however alexithymia is not the major factor that determines stress in CD. Nevertheless differences exist in the prevalence of three subtypes of this construct in TD1 and in CD patients. An early psychological therapeutic approach to the subject affected by TD1 showing high levels of alexithymia should be designed taking into account the three different subscales of the alexithymic construct.
Supported by Roche Diagnostics

0876

Needle phobia, anxiety, and self blood glucose monitoring

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Background and Aims: The management of diabetes often involves individuals testing their own blood glucose by capillary blood glucose measurement. This method is labeled "home" or "self" blood glucose monitoring and requires the use of a lancet device (finger prick) to obtain the appropriate aliquot of blood for glucose measurement. By contrast, needle phobia traditionally describes phobia to the insulin injection, rather than the finger prick testing method. There is accumulating evidence on phobia and anxiety relating to insulin injection, however to date there is limited evidence relating to phobia due to the finger prick method. The aims, therefore, of the present study were to assess the incidence of, and factors relating to, injection anxiety specifically relating to the finger prick method of measuring glucose.
Materials and Methods: Individuals with type 1 or type 2 diabetes, attending a London teaching hospital diabetes centre for routine follow up, participated in a self administered questionnaire on needle phobia and anxiety. In addition to demographic details, Injection Anxiety was assessed by using questions derived from the validated DSM-1V. General Anxiety was assessed by using the Anxiety subsection of the Hospital Anxiety and Depression Scale. Considerable Injection Anxiety was defined by an anxiety score>3 and high levels of General Anxiety was defined by a score>8. The following statistical methods were used for the data: Kendall's tau_b for rank correlations and Chi-square.
Results: Over a two month period 315 individuals completed the questionnaire: 93% reported previous self blood glucose monitoring of whom 50.0% test at least twice a day, 22.8% once a day, 19.7% twice a week, while only 7.5% less than once per week. Infrequent or complete avoidance of testing due to fear of the finger prick device was reported in 13.7% of the group. Nearly a quarter of the group (22.5%) reported other reasons (including - pain, forgetfulness, and time pressures) apart from needle phobia as reasons why they tested less frequently. Considerable Injection Anxiety (IAS>3) was observed in 30.2% and high levels of anxiety (GAS>8) was observed in 33.3%. Women compared to men reported higher Injection Anxiety and General Anxiety and ethnic minority groups reported

higher Injection Anxiety and General Anxiety. Significant positive correlations were found for avoidance of testing with Injection Anxiety and Injection Anxiety with General Anxiety. Significant negative correlations were found for age with General Anxiety and for age with testing times. Significant associations were found for gender with needle phobia, ethnicity with needle phobia, gender with anxiety, and for ethnicity with avoidance of testing.
Conclusion: A large proportion of the clinic patients appeared to have severe degrees of phobia and anxiety relating to the finger prick device which is more frequently reported in women and ethnic minorities. This observation is clearly important for the patient but also for the health care professional in identifying important management issues relating to self blood glucose monitoring.

PS 71

Education in diabetes

0877

Is patient education helpful in type 2 diabetes?
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Introduction: Systematic reviews indicate that diabetes education increases knowledge among people with type 2 diabetes, enhances positive changes in self-reported diet, reduces body weight and enhances metabolic control. However it is unclear which factors exactly contribute to these outcomes.
Aim: To analyze educational interventions in type 2 diabetes (T2DM) reported in randomized controlled trials (RCT) with regard to content, type and effectiveness.
Methods: Papers were extracted from Medline and Scopus from 2001 to October week 3, 2005 using e.g. educational intervention as keywords. Inclusion criteria were RCT design and adult T2DM. Papers regarding gestational diabetes and where results were not analyzed according to type of diabetes were excluded. WEKA data mining was used to explore the data.
Results: Of 464 titles extracted, only 19 articles met the inclusion criteria. The most common reason for exclusion was a non-interventional study. Data mining (WEKA) suggested that for initial HbA1c level≤7.9%, the intervention achieved only a small change in HbA1c level. For initial HbA1c≥8.0%, a significant drop in HbA1c level of 0.8% to 1.7% was observed. Data mining failed to identify duration, educational content or intensity of education as significant predictors of outcome.
Conclusion: Initial HbA1c level is the single most important factor predicting improvement in glycaemic control in response to patient education. The methodological details revealed in papers on patient education in T2DM is poor which hampers the utilization of results and dissemination of effective educational practices.

0878

Impact of a novel diabetes inpatient specialist nurse (DISN) service in the UK
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Background and Aims: A newly implemented DISN service was evakuated in order to calculate the number of patients seen, to assess the efficacy of and adherence to referral criteria, to identify whether the refferal was the result of a diabetes management incident and to determine the number of incidents encountered by the DISNs.
Materials and Methods: Data was collected on a proforma divided into 14 sections and a unique index of 34 diabetes incidents was used to obtain objective information.
Results: This study highlighted a vast workload for the DISNs, with a total of 890 patient visits across two hospital sites over a six month period. A large proportion of this workload involved the management of acutely ill patients with complicated diabetes. 308 patients were reffered with mean age of 64 yrs. 14% had Type 1 diabetes, 1.5% newly diagnosed and 68.5% had Type 2 diabetes, 16% newly diagnosed. 13% of patients with Type 2 diabetes required conversion to insulin therapy and education regarding self blood glucose monitoring. 33% of patients were referred due to acute illness resulting in unstable blood glucose levels, 10% because of hypoglycaemia, 14% following acute coronary syndrome, 5% active foot problems and 27% required education. Although 7% of referrals resulted directly from an incident, 36% of DISN visits identified one or more adverse incident. 63.5% of the incidents detected by the DISNs were attributed to insulin prescribing/administration errors resulting in preventable hypo/hyperglycaemia and the inappropriate treatment of hypo/hyperglycaemia.
Conclusion: Many diverse diabetes issues will go undetected without the DISNs' proactive approach. This study emphasises the importance of a proactive DISN service in identifying and managing common and preventable adverse incidents relating to diabetes inpatient care and providing

education and support for all non specialist health care professionals working in the hospital setting.

0879

The higher knowledege of diabetes, the better metabolic control and lower risk of microangiopathy in type 1 diabetic patients

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Background and Aims: The DCCT and its follow up EDIC study demonstrated that improvement of metabolic control and intensive insulin therapy reduces the risk of development and progression of late diabetic complications. The aim of this study was to assess the association between the knowledge of diabetes and intensive functional insulin therapy (IFIT) and prevalence of microangiopathy in type 1 diabetic patients treated with intensive functional insulin therapy from the onset of the disease.
Materials and Methods: 100 patients with newly diagnosed type 1 diabetes (43 female, 57 male, aged 24.1±6.1) were recruited to the study (between years 1994-1999). All subjects underwent a five-day teaching programme providing the skills in basal-bolus insulin management. We assessed at baseline and every one year: knowledge about diabetes and IFIT (test including 20 questions, maximum score: 20 points), number of hypoglycaemic episodes, quality of life, eye fundus, urine albumin excretion and parameters of metabolic control. The study group was divided into 3 subgroups according to scores achieved in the knowledge test: group A >16 points, group B: 12–16 points, group C: <12 points.
Results: During mean follow-up 7.1±1.5 years 18 (20%) subjects have developed diabetic nephropathy and retinopathy. Some results are presented in the table below.

At baseline	Group A	Group B	Group C
Knowledge (points)	17.4±0.9*	13.8±1.4	9.2±1.9
FPG (mmol/l)	8.6±3.9	9.8±3.2	11.1±4.1
2 h PPG (mmol/l)	8.1±4.2	9.9±4.2	11.1±5.6
HbA1c (%)	7.6±1.5*	7.7±1.6	9.2±1.9
LDL-cholesterol (mmol/l)	2.9±1.0	3.1±1.0	3.3±0.7
HDL-cholesterol (mmol/l)	2.0±0.3*	1.8±0.4	1.7±0.3
Triglycerides (mmol/l)	1.0±0.4	1.1±0.6	1.2±0.9
BMI (kg/m^2)	21.8±3.1*	23.5±2.6	23.9±3.2
SBP (mmHg)	120.0±15.9	124.9±16.4	127.5±15.6
DBP (mmHg)	75.7±8.0	76.5±9.3	78.6±7.7

*p<0.05 Group A vs Group C

We noticed higher risk associated with low diabetic knowledge (score:<12 vs>16 points) for microangiopathy (RR=3.71; 95%CI: 1.15–12.01, p=0.02)
Conclusion: The higher knowledge about diabetes at the baseline, the better metabolic control and lower risk for microangiopathy in Type 1 diabetic patients treated with intensive functional insulin therapy from the onset of the disease.

0880

The Poole diabetes education programme for newly diagnosed type 2 diabetes: an audit of outcome at 3 and 12 months

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Background and Aims: Group education for newly diagnosed type 2 diabetic patients has been established for over 25 years in the Poole area. It involves three 2-hour sessions over a 6 week period (Diabetes Education Session DES 1–3). The programme is facilitated by a diabetes nurse specialist and dietitian and involves a range of educational methods including interactive discussions, case studies and a food quiz. In 2002, we have updated the programme to encourage patient empowerment by involving patients in decision-making about diet and need for oral hypoglycaemic agents when reviewing home monitoring results and laboratory tests. We also started using community sites to run the programme making access easier for patients in the rural areas around Poole. After the education programme, the majority of patients are followed by the GP or practice nurse in primary health care. We undertook an audit to assess the effectiveness of the education programme and on going care for all the patients who attended in 1 year.
Materials and Methods: 897 patients attended the education sessions between 1 June 2003 and 31 May 2004. Using routinely collected data, we looked for any significant change in glycaemic control (HBA1c), cholesterol levels and weight between the first and the final session, and if this was maintained at 1 year.
Results: Following diagnosis, mean HBA1c at DES1 was 8.0% falling to 7.3% at DES3. Overall, mean HBA1c was 6.6% at 1 year. Mean reduction in HBA1c using the paired t-test was 0.8% (95% CI: 0.7, 0.9, p<0.001) between first and final sessions with a mean reduction of 1.4% (95% CI: 1.3, 1.5, p<0.001) at 1 year. Mean reduction in weight between DES1 and DES3 was 1.9 kg (98%CI: 1.7, 2.1; p<0.001). Mean reduction in cholesterol was 0.7 mmol/l (95% CI: 0.6, 0.9, p<0.001) from DES1 to 1 year. At DES3, 68% of patients achieved HbA1c <7.5% (primary

care QOF target) improving to 87% by 12 months. Our local target for patients is HbA1c <7.1% and 57% achieved this by DES3 improving to 77% at 12 months.
Conclusion: In newly diagnosed type 2 diabetic patients, our group education programme with emphasis on patient empowerment and patient involvement in clinical decision-making followed by on-going care in primary care achieved significant improvement in glycaemic control, weight loss and reduction in cholesterol levels.

0881

Effects of a structured education programme on illness beliefs, QoL and physical activity in individuals newly diagnosed with type 2 diabetes: the DESMOND pilot study

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Aim: Pilot study evaluating effects of the DESMOND structured education programme.
Method: 226 subjects newly diagnosed with T2D attended a DESMOND course in primary care, consisting of 6 hours of structured group education delivered to patients within 4–6 weeks of diagnosis, by 2 trained health professional educators. Patients completed measures of illness beliefs (IPQ-R, DIRQ) physical activity (IPAQ) quality of life (WHOQOL-BREF) prior to & 3, 6 & 12 mnths post attendance. Biomedical data collected at the same intervals.
Results: At 3 mnths subjects more likely to agree they understood their diabetes($t=-7.92;df=99;p<0.001$) that it is a serious health risk ($t=2.89;df=99;p<0.005$) a chronic illness ($t=2.09;df=99;p<0.05$), that they can affect its course $t=1.9;df=99;p<0.05$). Changes sustained at 6 & 12 mnths for perceived understanding, impact & duration of diabetes ($F=34.7;p<0.001;F=3.8;p<0.05$ $F=2.7;p<0.05$), at 6 mnths for perceived seriousness, personal responsibility($Fquadratic=10.0;p<0.005;F=44.8;p<0.005;Fquadratic=28.6;p<0.005$). Frequency of walking showed linear increase baseline to12 mnths ($Flinear=7.0;p<0.05$). Physical domain of QoL increased baseline to 3 mnths ($t=2.03;df=131;p<0.05$) sustained at 12 mnths. Complete biomedical data in 60/109 patients showed significant reduction in A1c($p<0.01$)BP ($p<0.01$)weight($p<0.05$)& waist circumference($p<0.05$) baseline to12 mnths, and in lipids baseline to 6 mnths ($p<0.01$). Subjects agreeing more strongly they understood their diabetes($\beta=.18;p<0.05$)and that it is a chronic illness ($\beta=.20;p<0.05$) reported greater increase in frequency of physical activity. Greater belief in understanding diabetes ($r=.21;p<0.05$) the better QoL. Greater belief in ability to control diabetes($r=.24;p=.05$)greater A1c reduction.
Conclusion: Pilot data indicates the DESMOND course facilitates changes in illness beliefs, sustained at follow-up and predictive of metabolic control. A full scale randomised controlled trial is now underway in 17 primary care organisations and 238 practices in England and Scotland. Recruitment is completed with 823 patients randomised, and is due to report early 2007. HbA1c is the primary outcome.
Supported by Diabetes UK

0882

Changes in dietary practices of type 2 diabetic subjects during Ramadan fasting

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Background and Aims: Fasting during the month of Ramadan is one of the most important religious practices of millions of Muslims in the world. Ramadan fasting involves major changes in dietary pattern and metabolic state. Dietary measures comprise the first line therapy for controlling metabolic changes during Ramadan. The aim of the study was to explore the dietary practice of Bangladeshi type 2 diabetic subjects during Ramadan Fasting.
Materials and Methods: Ninety two type 2 diabetic subjects (male: female ratio 61:31, age 47±9 years, mean±SD) were selected from the Out-Patient Department of BIRDEM (the tertiary care hospital of Diabetic Association of Bangladesh). The first survey was conducted 1–4 weeks before Ramadan and the second interview was done during 3–4 weeks of Ramadan. The dietary intake of macro- and micronutrients by the study subjects was calculated by a specific food frequency questionnaire (FFQ). Data were expressed as Mean+SD. Difference between during Ramadan and before Ramadan values were calculated using paired t-test. Spearman's coefficient correlation analysis was performed for skewed data.
Results: BMI of the study subjects was significantly reduced during Ramadan compared to before Ramadan (24.5+3.4 vs 24.1+3.4, Mean+SD, $p<0.001$). No significant difference was found between during and before Ramadan in terms of per day intake of total energy (calorie, 2424+

678 vs 2418+1137) carbohydrate (g, 363+128 vs 385+205), protein (g, 95.68+42 vs 97.1+69.9) and fiber (g, 6.2+2.4 vs 5.9+3.2) among the study subjects. However, daily fat intake was significantly higher during Ramadan compared to before Ramadan (g, 65.61+15.67 vs 54.5±15.3; $p<0.05$). In terms of proportion the fat intake increased by about 26% during Ramadan. The major source of this increased fat was traditional oily foods. A positive correlation was found between total cholesterol and fat intake ($r=0.046$, $p=0.673$) during Ramadan. Iron and calcium intake were found significantly higher during Ramadan compared to before Ramadan (mg/day, 31.89±17.83 vs 25.3±14.06 and 747±469 vs 588±349, respectively, $p<0.05$). Total thiamin intake of the subjects was significantly lower during Ramadan compared to before Ramadan value (mg/day, 1.72±0.91 vs 2.11±1.75, $p<0.05$). But riboflavin, vitamin-C, beta-carotene and retinol intake remained unchanged during Ramadan.

Conclusion: In Bangladeshi population, Ramadan fasting is accompanied by weight loss without any change in overall energy balance indicating a possible dehydration in this condition. Substantially increased fat intake during Ramadan fasting may lead to dyslipidemia and it warrants educational efforts among the population to promote a balanced and healthier diet during the Holy Month.

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0883

Meta-analysis do not allow appraisal of education programmes of diabetes or hypertension self-management - a systematic review

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Background and Aims: Patient education programmes are complex interventions. Their evaluation is difficult because of problems in identifying and assessing the effects of their various components. The U.K. Medical Research Council proposed a framework for the design and evaluation of complex interventions. Five sequential phases of 'increasing evidence' have been defined including development, efficacy and implementation trials. The aim of the present review was 1) to systematically explore problems of current methodologies in systematic reviews that evaluate patient education programmes in the field of diabetes care and hypertension; and 2) to propose a systematic approach allowing an appropriate evaluation.

Materials and Methods: A systematic search has been performed in the Cochrane Database, DARE, PubMed, Cinahl (3/2006) and HTA-databases (11/2005). The selected reviews were analysed by two independent investigators using standard extraction forms. It was analysed 1) if the 'increasing evidence' was detected and included; 2) if the active components were identified and analysed; 3) if and why the programmes had been allocated into categories of interventions; 4) if all relevant outcomes had been assessed and if interactions between them had been analysed.

Results: Our data base searches yielded 1441 results. All titles were screened; 1311 publications were excluded, they were not systematic reviews or not relevant. The remaining 130 abstracts were assessed for inclusion. A total of 15 reviews in 16 publications were considered eligible. Meta-analysis was performed in 8 reviews; 7 reviews used qualitative data-synthesis. In 6 reviews only controlled trials were included; other types of publication concerning the 'increasing evidence' were not considered. In 8 reviews experts in the field were contacted. Only one review reported author contact. Theoretical funding was discussed in 10 reviews. 12 reviews reported that the included programmes had been multidimensional. The impact of active components was analysed in 10 reviews. The approach of 'increasing evidence' was cited in 3 reviews. Interdependencies between outcome measures were considered in 3 reviews.

Conclusion: Most of the included reviews discussed methodological challenges to appraise complex interventions. However, these considerations did not have any impact on the methods used. We therefore propose the following approach: 1) all studies referring to the phases of 'increasing evidence' should be considered; 2) searching should not be limited to certain types of studies, authors should be contacted; 3) interdependencies between the active components should be taken into account, components should only be examined separately if they are independent and should not be disassembled if they work interdependently; 4) education programmes should not be allocated into categories referring to interdependent components; 5) all relevant outcome parameters should be included; 6) pooling of outcome measures across different programmes is inappropriate; the interdependency between treatment goals and outcomes has to be considered. Information necessary for the evaluation of education programmes is difficult or impossible to identify. Therefore, we propose to create an electronic database which provides available programmes together with all relevant background information or to implement such a system into existing databases.

0884

Improving diabetes education using Alphabet Strategy. Use of printed educational materials versus electronically stored information. The GEDI Project (*G*eorge *E*liot *D*iabetes *I*nformation *P*roject)

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Background and Aims: There is an increasing need for effective education in diabetes to empower the patient to better self manage their own care. Traditionally printed material is used but can be expensive to produce. Now electronic devices are becoming cheaper
Our aim was to compare the effectiveness of using computer assisted learning using a portable memory stick and printed form.
Materials and Methods: A baseline survey of 100 patients revealed that there was most interest in knowing about the following (scores out of 4 maximum):
• Diabetes Control (3.52)
• Risk factors (3.54)
• Emergency situations (3.36)
82% of patients expressed an interest in having copies of their clinic letters with 49% expressing a very strong interest. 100 patients were included in the study. All patients had to have access to a computer and agree to participate in this project. Participants were requested to complete a questionnaire on diabetes, risk factors for complications and daily management. This is based on the Alphabet Strategy: ***A***dvice (diet, exercise, smoking cessation, concordance with treatment), ***B***lood pressure (<145/85 mmHg), ***C***holesterol (≤5 mmol/l), ***D***iabetes control (<7.5%), ***E***ye care and ***F***oot care (annual screen and treatment), ***G***uardian drugs, e.g., Aspirin, ACE-I. There are 35 questions with multiple choice and short answer format. Previously we have shown the Alphabet Strategy to be enthusiastically accepted by patients as a method of education.
Patients were then randomised to receive educational materials on a portable memory device (n=50, GEDI, George Eliot Diabetes Information stick) or in printed format (n=50). GEDI contained information including their clinical letters (last 3 years), their diabetes care plan and an overview and summary of the Alphabet Strategy. The paper format had exactly the same materials. The GEDI device costs €2.60 versus €8 for the paper format.
Results: The average age of the patients with was 37.1 (range 14–76). This included patients with type 1 diabetes or type 2 diabetes. Baseline education score was 61.5%±22.1% in the GEDI group versus 61.5%±20.7% in the paper format group (NS). After a period of 12 to 16 weeks there was a significant improvement in education scores in both groups (GEDI to 80.0%±15.8%. Paper format 84.7%±16.3%) ($p<0.001$). There was no difference in increase in improvement comparing the two different methods. However, only 31/50 returned the second questionnaire and 23/50 in the paper format group. After a cross over-period, when all patients had both GEDI and the paper format, the education scores remained stable at above 80% in both group
Conclusion: Computer assisted learning using a portable memory stick was as effective as the more expensive printed materials. We also demonstrated a significant improvement in education scores amongst our patients using the locally-created but freely available Alphabet Strategy.

0885

Field glucose variability index is related to laboratory measures of insulin sensitivity and hypoglycaemia counterregulation

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Background and Aims: Contemporary studies increasingly support the idea that blood glucose (BG) variability is a major determinant of diabetes complications; therefore BG variability, along with HbA_{1c}, becomes a major marker of glycemic control in Type 1 and Type 2 diabetes (T1DM, T2DM). However, quantifying glucose variability is not straightforward - traditional variability measures, such as standard deviation (SD), M-value, etc. appear to be mostly dependent on hyperglycemic excursions, ignoring hypoglycemic BG fluctuations and being insensitive to hypoglycemia-related characteristics of T1DM.This study introduces a new variability measure computed from routine self-monitoring BG (SMBG) data, the Average Daily Risk Range (ADRR), which is equally sensitive to hypoglycemic and hyperglycemic excursions, and investigates its relationship with laboratory parameters of glucose dynamics in T1DM.
Materials and Methods: Twenty-five adults with T1DM, average age=42.5±12 years, duration of diabetes= 21.6±9.4 years, HbA_{1c}=7.4±0.8, 16 males, performed routine SMBG for 2 months, 4–5 times/day. The ADRR was computed from routine SMBG data taking into account the value and the time of each SMBG reading. SD of BG and M-value were computed as well. Subjects were then hospitalized and their BG was controlled overnight at ~6 mmol/l. Hyperinsulinemic clamp (1 mU/kg/minute) was initiated in the morning, beginning with 2-hour euglycemia at ~5.5 mmol/l, followed by 1-hour descent into hypoglycemia with a target level of 2.2 mmol/l. BG

was sampled every 5 minutes (Beckman glucose analyzer); epinephrine was sampled twice at euglycemia and every 10 min during descent in hypoglycemia. Glucose uptake during euglycemia was computed in mg/kg/min as a marker of insulin sensitivity.
Results: Within the field SMBG data the ADRR correlated approximately equally well with significant hypoglycemic (BG<2.2 mmol/l) and hyperglycemic (BG>22.2 mmol/l) episodes, correlations of r=0.42 and r=0.51 respectively, p<0.05. In contrast, the SD of BG and the M-value had zero correlation with hypoglycemic events and r=0.63, r=0.54 with hyperglycemic events respectively, confirming previous observations that these measures are primarily related to hyperglycemic excursions. The ADRR, computed from SMBG, was highly predictive of subsequent hospital laboratory results: its correlation with subjects' glucose uptake during euglycemia was 0.59; its correlation with [the logarithm of] peak epinephrine during induced hypoglycemia was −0.59, p=0.002, indicating that the ADRR was equally related to both insulin sensitivity and degree of counterregulation. No other measure of glucose variability achieved statistically significant relationship with these hospital parameters.
Conclusion: A new field glucose variability measure called ADRR that is calculated from regular time-stamped SMBG data, specifically designed to be equally sensitive to hypoglycemia and hyperglycemia, is more closely related to both increased glucose uptake and reduced counterregulatory response than any other measures of variability tested. Further studies will determine whether the active reduction of field glucose variability, as measured by ADRR, will improve counterregulatory response and insulin sensitivity in T1DM.
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0886

The periodontium condition in men with type 1 diabetes
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Background and Aims: Diabetes has been implicated in the development of significant periodontal disease and with other significant local effects on the oral cavity that contribute to periodontal disease. The aim of thus investigation was to relate the effects of the metabolic control of adult type 1 diabetes to the periodontal health of these patients. It has been suggested that long-term poorly controlled insulin dependent diabetic (PIDD) subjects are at risk for periodontal disease. We compared the periodontal health status of PIDD subjects with controlled insulin-dependent diabetic (CIDD) subjects in cross-sectional and longitudinal studies.
Materials and Methods: At basic examination, 18 PIDD, 20 CIDD and 20 subjects of controls were examined. The subjects were male, mostly long-term (>10 years) type 1 diabetics with an age range from 18 to 40 years. Mean glycosylated hemoglobin and blood glucose levels were significantly higher in the PIDD (HbA1c≥9%) than the CIDD (HbA1c − 6,5–7,5%) subjects at baseline (p<0,001).
Results: The one-year longitudinal study revealed that the PIDD subjects showed greater tooth loss, more gingival inflammation and bleeding after probing than did the CIDD subjects. Despite similar oral hygiene conditions, at the first and at the two subsequent examinations the PIDD subjects exhibited more gingival recession than the CIDD subjects (p<0,05), more loss of attachment (p0,05).
Conclusion: These data may suggest that poorly controlled diabetes accelerates the progression of periodontitis lesion in the active phase of the disease.

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Social and economic implications of diabetes treatment

0887

Trends in outpatient prescription drug costs in diabetic patients in Germany, 1994–2004
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Background and Aims: New antidiabetic medications have been introduced during the last decade, but their impact on health care cost is largely unknown. Therefore, prescription costs in diabetic patients and nondiabetic controls in primary care were evaluated (Germany: 1994–2004).
Material and Methods: Data were derived from a nationwide pharmaceutical database (IMS Health, Disease Analyzer). Primary care data of all 46,017 diabetic patients and 46,017 controls in 417 nationwide practices (2004) were compared to 29,956 diabetic patients and 13,226 controls (361 practices) in 1994. Age- and sex-standardised prescription prevalences, costs and cost ratios (2004/1994), using drug company sales prices, adjusted for inflation (German Consumer Price Index) were calculated.

Results: A 50% prevalence increase for insulin prescriptions (28.9% vs 19.3%), a 4fold increase for biguanide use (35.2% vs 9.4%), and a threefold higher prevalence of blood glucose self-monitoring prescriptions (BGSM) (28.7% vs 9.9%) was observed over 10 years (all p<0.01). A decline was found for sulphonlyureas (−37%) and acarbose (−50%). Mean total annual drug costs (company sales prices) per diabetic patient were 523 Euro in 2004 (1994, inflation-adjusted: 335 Euro) equalling a 56% increase (p<0.01). Average costs for antidiabetic medication were 192 Euro in 2004, a 100% increase (p<0.01). The major antidiabetic cost factors were insulins and analogues (mean costs per patient, 2004 vs 1994: 113.9 vs 58.1 Euro, Ratio: 2.0, p<0.01), followed by oral antidiabetics (44.0 vs 20.9 Euro, 2.1, p<0.01), and BGSM (33.9 vs 14.8 Euro, 2.3, p<0.01). In 2004, insulins accounted for 22% of total drug costs among diabetes patients (1994: 17%), all oral antidiabetics combined captured 8.4% (1994: 6.2%), and BGSM accounted for 6.5% (1994: 4.4%). Although glitazones were only prescribed in 3.6% in 2004, average expenses (12.0 Euro) in the entire diabetic population were already comparable to biguanides (10.9 Euro) and sulphonylureas (9.8 Euro) due to its high annual treatment costs (332 Euro per treated). Overall, newly launched antidiabetics (insulin analogues, glitazones, glinides) accounted for 40% of antidiabetic drug costs in diabetic patients in 2004. The 38% increase (331 vs 240 Euros) in non-diabetes related drugs over the 10-year period among diabetic patients was mainly due to cardiovascular drugs. In nondiabetic patients, a comparable drug cost increase (33%) was found (222 vs 167 Euro, p<0.01).

Conclusions: Prescription drug costs among diabetic patients in primary care in Germany increased about 60% during the last decade, which was about twofold higher than in nondiabetic patients. In particular, an overproportional increase (100%) was found for costs of diabetes treatment (insulin, oral antidiabetics) and blood glucose self-monitoring test. New types of antidiabetic drugs accounted for a substantial cost share in 2004. Thus, pharmacological advance is a key driver of drug expenditure growth in diabetes care, indicating the need of cost-effectiveness analyses of new agents.

0888

Ambulatory HbA_{1c} determination is cost-effective in the fol low-up of type 1 diabetic patients

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Background and Aims: Although ambulatory HbA_{1c} determination is preferred by most patients and reduces their time wasted for sampling, it is unknown if this technique is also cost-effective when indirect costs are taken into account. The aim of the study was to compare direct and indirect costs between two different follow-up strategies of type 1 diabetic (T1D) patients.

Materials and Methods: 87 T1D patients were randomized in a 6-month prospective study in two groups using either (1) ambulatory HbA_{1c} determination by means of a DCA 2000 meter (ambulatory, AMB group, Bayer Diagnostics) or (2) central laboratory HbA_{1c} determination (control, CON group, HPLC) in addition to other routine biochemical parameters (glucose, creatinine, uric acid, lipids, AST and ALT). CON patients were instructed to estimate the working-time lost due to traveling, waiting and sampling time in the hospital as well as public transportation costs, both at 3 and 6 months. The costs of time lost for each patient (indirect costs) were evaluated using individual income data or alternatively the minimum salary for not active-working patients (2004, Spain). The estimated sample to achieve a statistical difference was 41 patients per group with a 0.05 alfa and a 0.1 beta error, considering 10% of patient lost in the follow-up, a 5.00 € standard deviation and a 4.02 € difference to be detected. Statistical analysis was made using the t-Student test.

Results: 42 T1D patients were included in the AMB (age 33±8 years-old, 20 women, BMI 24.9±3.8 kg/m^2, diabetes duration 12±9 y, basal HbA1c 7.4%) and 45 in the CON group (age 37±12 years-old, 14 women, BMI 24.1±2.6 kg/m^2, diabetes duration 16±11 y, basal HbA1c 7.7%). Educational level and mean income were comparable between the groups. In comparison with the HPLC method, we found that ambulatory HbA_{1c} determination was+2.50 € more expensive (direct costs). Working time lost due to sampling in the hospital was 80±36 min in the control group. Total costs (direct+indirect costs) were 6.90 € (95% CI, 5.90–8.00 €, p<0.0001) higher in the CON vs AMB group.

Conclusion: Ambulatory HbA_{1c} determination is a cost-effective approach when used in T1D patients. This strategy is associated with a diminution of indirect and total costs and, additionally, may help patients to save an important amount of time due to sampling prior to medical follow-up visits.

0889

The association of socioeconomical status and the risk of diabetes type 2 estimated by Finnish Diabetes Risk Score criteria in Polish population

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Background and Aim: The socioeconomic status (SES) has the impact on many lifestyle factors like physical activity, obesity and nutritional patterns. The association of SES with elevated blood glucose and diabetes type 2 risk assessed by Finnish Diabetes Risk Score (FRS) has not been well studied yet.
Aim: The aim of the study was to examine the relation between the socioeconomic status and and the risk of diabetes type 2 according to FRS criteria.
Sample and Methods: The studied population were 977 men and 983 women aged 45–64, randomly selected from the Krakow's population. All analyses were performed after exclusion of subjects with known diabetes. Education level, marital status, professional position and economical status data were collected from the standardized questionnaire. Chi square test was used for estimation of relation between the SES in FRS criteria and elevated blood glucose.
Results: The FRS below 7 was present in 33.2% men and 25,9% of women ($p<0,05$)), and the FRS between 15–20 was present in 11,8% men and 15,6% of women ($p<0,05$)). University education had 30,2% men and 26,0% women. The risk of having diabetes estimated by FRS is lower in university educated women compared to less educated group. The same observation was found for elevated blood glucose. There were no assotiation between FRS and elevated blood glucose with education in men and with other SES variables in both sex.

		Education level Elementary and high school	high school
Findrisk score >7	Men	67,9%	64,1%
	Women*	77,8%	63,7%
Glucose >6,1 mmol/l	Men	16,9%	16,8%
	Women*	11,0%	6,0%
Glucose >7,0 mmol/l	Men	4,5%	4,8%
	Women*	3,8%	0,9%

*$p<0,05$

Conclusion: The higher education was related to lower risk of type 2 diabetes according to FRS criteria in women but not in men. The same was observed for elevated blood glucose. This findings might confirm the impact of education on lifestyle factors associated with development of diabetes in women found in previous studies.

0890

Assessing the economic burden of needlestick injuries associated with insulin delivery amongst healthcare professionals in community settings in the UK

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Background and Aims: Needlestick injuries to healthcare professionals represent an avoidable economic burden to the health service and can cause significant anxiety and distress to healthcare workers. The National Audit Office suggests that such injuries are the second leading cause of staff absence in the NHS in the UK. Research has identified that the use of unfamiliar sharps and the removal or re-sheathing of needles increase the risk of injury. Healthcare workers who administer insulin may be at elevated risk as they often use a patients' own insulin delivery device. Research involving healthcare professionals estimated that between 10–20% of all needlestick injuries are associated with insulin delivery. This research aimed to quantify the burden of needlestick injuries to healthcare professionals associated with insulin delivery in community settings in the UK.
Materials and Methods: Data on the number of people with diabetes in the UK, the rate of third party insulin delivery in the community, the rate and cost of needlestick injuries were drawn from published sources. Where data were unavailable from the literature, assumptions based on expert input were applied. Simple modelling techniques were used. No account has been made to take into account injuries that occur in hospital settings. As such, any estimates should be regarded as conservative
Results: Current estimates suggest that there are 1.8 million people diagnosed with diabetes in the UK. Approximately 25% of these are believed to be insulin dependent. Our model assumes that 2% of insulin dependent diabetics rely on a third party for insulin administration.
Based on a previously published study, the average number of injections per day is estimated to be 1.68 per person. The rate and cost of needlestick injuries are drawn from studies conducted by the Scottish Executive. The rate of injuries used is 250 per million needles. Based on published research, an average weighted cost of £362 per needlestick injury was derived. This covers all post-exposure management including prophylaxis, laboratory testing, counselling and where necessary treatment. Based on the above data, our analysis estimates the total burden to the NHS of needlestick injuries associated with insulin delivery in the community to be £499,614.
Conclusion: Needlestick injuries associated with insulin delivery in community settings are estimated to cost the NHS almost £500,000 per annum. This takes no account of

under-reporting or injuries occurring in other care settings and is likely to be an under-estimate of the true cost. The analysis also takes no account of the costs which may be associated with litigation which is increasing.
Many of these injuries are avoidable through improved professional practice or the use of safety devices designed to reduce the risk of injury. Best practice guidelines incorporating recommendations on the use of safety devices should be promoted to reduce this unnecessary burden on health care workers and NHS resources.
Supported by Novo Nordisk

0891

Continuous subcutaneous insulin infusion versus multiple daily injections for type 1 diabetes: a cost-effectiveness analysis for Sweden

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Background and Aims: A recently published meta-analysis of insulin pump therapy in patients with diabetes identified some important differences between continuous subcutaneous insulin infusion (CSII) and multiple daily injection (MDI). The analysis took into account 52 studies and over 1,500 patients and reported that CSII was associated with improved HbA1c, compared to MDI. The aim of this study was to project the long-term clinical and cost outcomes of CSII versus MDI in patients with type 1 diabetes in Sweden, based on this meta-analysis.
Material and Methods: The CORE Diabetes Model, a published and validated, interactive computer model was adapted to simulate the long-term incidence and progression of diabetes-related complications in the Swedish setting. The model utilized Markov modeling and Monte Carlo simulation techniques, and disease progression was simulated in a hypothetical cohort of patients with type 1 diabetes and baseline characteristics defined in terms of demographics and management and complication status. Baseline cohort characteristics included mean age 27 years, duration of diabetes 6 years and mean HbA1c 8.875%. Basecase intervention effects were based on a meta-analysis by Weissberg-Benchall *et al*. who reported a mean reduction in baseline HbA1c of 1.2%-points with CSII compared to MDI. This intervention effect was set to 0.51%-points in a sensitivity analysis, according to data reported by Pickup *et al*. Costs were accounted from a societal perspective (treatment, complication and lost-productivity costs) and expressed in year 2005 Swedish Kroner (SEK) values. Clinical and cost outcomes were projected over a lifetime horizon and discounted at a rate of 3% *per annum*.
Results: In the basecase, treatment with CSII was associated with improved mean life expectancy (0.844 life years) (17.55±0.21 versus 16.71±0.20) and quality adjusted life expectancy (QALE) (0.850 quality-adjusted life years [QALYs]) (12.06±0.14 versus 11.17±0.13), compared to MDI. Mean direct lifetime costs were SEK 348,582 higher with CSII treatment compared to MDI, but incremental total lifetime costs were offset to SEK 193,078 for CSII versus MDI after indirect costs were accounted; CSII was assumed to be associated with greater workplace productivity through fewer diabetes-related complications. This produced an incremental cost-effectiveness ratio (ICER) of SEK 227,066 (~€24,300) per QALY gained with CSII versus MDI. In the sensitivity analysis, using the results from Pickup *et al.*, the relative outcomes were unchanged, with CSII associated with increased life expectancy (0.370 life years) and QALE (0.505 QALYs) compared to MDI, with an ICER of 482,920 (~€51,600) per QALY gained.
Conclusions: CSII was associated with increased life expectancy and QALE, and a reduced incidence of diabetes-related complications, compared to MDI. ICERs indicated that CSII represented good value for money from a societal perspective by current standards in Sweden, compared to MDI.
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0892

Evaluating improvements in life expectancy and diabetes-related complications associated with reaching HbA_{1c} targets: a modelling study of type 2 diabetes in the UK setting

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Background and Aims: To determine the long-term health economic impact of treatment to target HbA1c levels in patients with type 2 diabetes in the UK.
Materials and Methods: A validated and published model for type 2 diabetes was used to estimate the impact of treating patients to target HbA1c levels on complication rates, life expectancy (LE), quality-adjusted life expectancy (QALE) and complication costs in a 'typical' UK cohort of

type 2 diabetes patients. Four hypothetical reductions in mean HbA1c were simulated (versus no reduction): 1) 12% to 9.5%; 2) 9.5% to 8%; 3) 8.0% to 7.0%; and 4) 7.0% to 6.5%. All other clinical parameters (blood pressure, body weight and lipid levels) remained constant. Patient characteristics were derived from the HODaR database which included over 20,000 patients with type 2 diabetes in England and Wales. Complication costs, inflated to 2004 British pounds (£) were discounted at 3.5% annually in line with NICE recommendations.

Results: In poorly controlled patients, decreasing HbA1c from 12% to 9.5% delayed the onset and reduced the cumulative incidence of complications, particularly retinopathy and nephropathy, increased LE and QALE, and reduced costs of treating diabetes-related complications. Improvements were also projected in well controlled patients (reduction from 7.0% to 6.5%). Sensitivity analysis showed these results to be consistent when variation in the time horizon and discount rates were applied.

Conclusion: It is well established that improving glycemic control improves outcomes. Our study shows that improving HbA1c leads to substantial clinical and economic benefits (increased in LE, QALE and a reduction in the costs of treating diabetes-related complications) even in patients who are not close to recommended HbA1c targets, supporting the hypothesis that any improvement in HbA1c is beneficial in patients with type 2 diabetes.

Summary of Clinical and Cost Outcomes

	Undiscounted LE (years)	Difference (HbA1c reduction - no reduction) Undiscounted QALE (QALYs)	Lifetime cost of complications (£)
Scenario 1 (12.0 to 9.5%)	1.24	0.91	−3,946
Scenario 2 (9.5 to 8.0%)	0.65	0.50	−1,802
Scenario 3 (8.0 to 7.0%)	0.45	0.35	−947
Scenario 4 (7.0 to 6.5%)	0.19	0.15	−443

Supported by Novo Nordisk Ltd., UK

0893

External validation of the economic assessment of glycaemic control and long-term effects of diabetes (EAGLE) model

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Background and Aims: The Economic Assessment of Glycemic Control and Long-term Effects of diabetes (EAGLE) model version 2.0 was developed to provide a flexible and comprehensive tool for the simulation of the long-term effects of diabetes treatment and related costs in type 1 and type 2 diabetes. EAGLE simulations are based on risk equations, developed using published data from several large studies including the Diabetes Control and Complications Trial, the United Kingdom Prospective Diabetes Study, and the Wisconsin Epidemiology Study of Diabetic Retinopathy. We previously demonstrated that EAGLE is an internally valid model; EAGLE was able to reproduce the results of the published studies used to build the model within an acceptable range of±10%. The aim of the present study was to demonstrate the EAGLE model's ability to predict the results of studies that were not used to build the model, using standard methodologies for performing external validation.

Materials and Methods: External validation included the following steps: 1) A systematic literature search to identify suitable publications related to glycemic control. Publications were rejected if they did not contain sufficient details of the main influence parameters (HbA_{1c}, systolic blood pressure, age, and gender) and if they did not define events in a similar way to the definitions used in EAGLE; 2) Simulation sets were created in EAGLE based on the data contained in the selected articles. Patient numbers were set as 50,000 and iterations as 5,000; 3) Simulations were run. The results obtained with EAGLE were compared with the outcomes reported in the selected publications.

Results: Thirteen publications met the inclusion criteria and were selected for the external validation. Our analyses demonstrated that every mean event rate simulated by EAGLE deviated from the published event rates within an acceptable range of 0% to 30%, and always overlapped with the confidence intervals of the published event data. For example, in type 2 diabetes, EAGLE successfully predicted the heart failure event rate with only 6% deviation in comparison to rates published by Torffvit and Agardh (J Diabet Comp. 2001;15:307–313).

Conclusion: The EAGLE model is an externally valid tool for making predictions about the long-term effects of diabetes treatment in type 1 and type 2 diabetes patients. The external validation of EAGLE showed that the model is able to predict event rates, reported in studies that were not used to build the model, within a reasonable range of deviation.

Supported by Pfizer Inc

0894

Health-related quality of life and costs of receiving add-on therapy with insulin glargine or pioglitazone in patients with type 2 diabetes

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Background and Aims: Diabetes and its treatment can impact health-related quality of life (HRQoL). We examined HRQoL in patients with type 2 diabetes who received insulin glargine (GLAR) versus pioglitazone (PIO) as add-on therapy in a randomized clinical trial. Additionally, we assessed the economic cost associated with these therapies.
Materials and Methods: Patients, inadequately controlled on sulfonylurea or metformin (MET), were randomized to GLAR (n=189) or PIO (n=199) for 24 weeks of treatment, and follow-up was subsequently extended to 48 weeks. HRQoL was assessed at baseline and each of 10 follow-up visits, with the 34-item Diabetes Symptom Checklist-Revised (DSC-R), the Emotional Well-Being, and General Health Perceptions scales from the Medical Outcomes Study 36-item Short-Form Health Survey. Using the DSC-R, patients were asked to indicate whether or not they experienced a particular diabetes symptom, such as numbness in the feet, and to rate the intensity of that symptom from 1 (not at all) to 5 (extremely). The cost calculation included components of study medication, other antidiabetic agents, syringes, test strips, and lancets for self-monitoring of blood glucose, and liver function test costs according to PIO label. Average wholesale price was used for drugs and supplies; costs of liver function tests were determined using fee schedules from Medicare's Resource-Based Relative Value Scale. The frequency of self-monitoring of blood glucose was based on the study protocol.
Results: The average daily dose was 47 IU for GLAR and 33 mg for PIO. GLAR provided better glycemic control compared with PIO [mean A1C change from baseline: −2.6% (GLAR) vs −2.3% (PIO); $P \leq 0.05$]. 230 subjects completed the baseline and week-48 HRQoL assessments (n=118, GLAR; n=112, PIO). GLAR had greater improvement in 17 of 20 HRQoL measurements from baseline to week 48 relative to PIO; findings were statistically significant ($P<0.05$) for the domains measuring psychological distress related to hyperglycemic symptoms, fatigue, and total symptom-related distress. In multivariate repeated-measures analysis, GLAR was associated with significantly better outcomes for hyperglycemic ($P \leq 0.001$) and ophthalmologic symptoms ($P=0.004$) and with psychological distress related to fatigue ($P=0.005$), hyperglycemic ($P<0.001$), hypoglycemic ($P=0.014$), ophthalmologic ($P<0.001$), and cardiovascular symptoms ($P=0.025$). The estimated mean total cost of glycemic control over 48 weeks was $379 lower for insulin glargine subjects ($1721 vs $2100 for pioglitazone).
Conclusions: Insulin glargine, when added to oral monotherapy, resulted in improved glycemic control and diabetes-related quality of life with lower costs compared with pioglitazone.
Supported by sanofi-aventis U.S.

0895

Cost effectiveness of SMBG for the treatment of non insulin dependent diabetes mellitus

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Background and Aims: Despite a number of randomized controlled trials having been conducted to assess the value of SMBG in Type 2 diabetics not using insulin, controversy remains. Whilst it would be premature to restrict use of SMBG (self measurement of blood glucose) based upon current evidence, a clear appreciation of the cost associated with use of SMBG is required before any decision on interim policies can be made. Reports on the cost effectiveness of SMBG are lacking.
Materials and Methods: A Markov model of diabetes management and complications was used to perform a cost effectiveness analysis of SMBG in non-insulin requiring type 2 diabetics treated over a 10 year period.
We assumed that the sole impact of SMBG was on glycaemia as reflected by changes in HbA1c. The impact of SMBG on HbA1c was extacted from the recent meta-analysis of Sarol et al. (Curr.Med.Res.Opin., 2005) in which both random and fixed effects estimates were calculated for a frequency of testing of 5–7 times per week.
Results: At a frequency of SMBG testing of 7 times per week and assuming a reduction in HbA1c of 0.42% the CLYG (cost per life-year gained) was within the limits of acceptability with both Metformin and sulphonylurea treatment, being respectively €28,171 and €27,062. The reduction in diabetes related complications was of greatest clinical impact in retinopathy and nephropathy for which reductions of between 10–15% were calculated. To test the impact of variable patient responsiveness to SMBG we recalculated the associated costs when the change in HbA1c approached the lower and upper confidence limits defined in the meta analysis of Sarol et al. The result of this was a change in CLYG to €63,404 and €19,351 for the lower and upper limits respectively.

Conclusion: We have shown in our analysis that if the recently reported and significant 0.4% reduction in HbA1c calculated by pooling data from recent RCT studies is assumed, then SMBG is cost effective. Using a Markov model of diabetes and its complications we determined that a reduction in HbA1c of 0.4% would result in modest improvements particularly in the event rates for retinopathy and nephropathy in the order of 10–15%, and that the cost of achieving this via SMBG was within the economic limits of acceptability, at a CLYG of €28,171.
Supported by sanofi-aventis U.S.

0896

Higher medical care costs accompany the IDF metabolic syndrome

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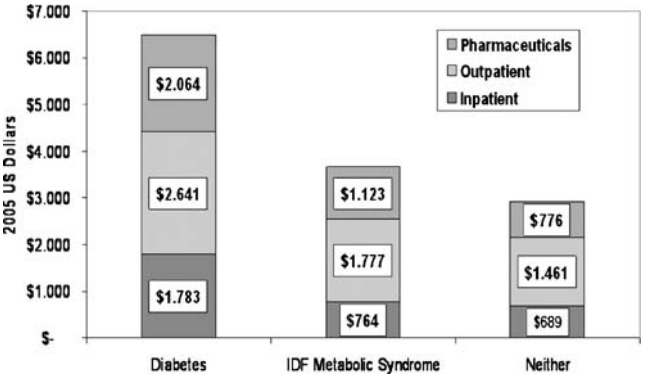
Background and Aims: The EASD and ADA recently issued a joint statement calling for a critical appraisal of the metabolic syndrome. We sought to contribute to that appraisal from an economic perspective. Specifically, our objective was to compare costs among patients with 1) type 2 diabetes; 2) no diabetes but who otherwise met International Diabetes Federation (IDF) criteria for the metabolic syndrome; and 3) neither diabetes nor IDF metabolic syndrome.
Materials and Methods: We identified 33,054 members of a large group model HMO located in the Western United States who, between 1 July 2003 and 30 June 2004, had all clinical measures necessary to ascertain IDF metabolic syndrome status. However, waist circumference was not available, so we substituted body mass index (BMI) for the central obesity criterion, using a cut-point of 27.0 kg/m^2. Subjects with a diagnosis of type 2 diabetes (n=8,573) were assigned to the diabetes group regardless of metabolic syndrome status. Of the remainder, 10,419 met IDF metabolic syndrome criteria, and 14,062 did not. We calculated and compare age-and sex-adjusted medical care costs (in 2005 US dollars) among these three groups for the one-year period of July 2004 through June 2005. In addition, we estimated a multivariable model regressing total costs on age, sex, and dichotomous variables for each of the five metabolic syndrome criteria among patients without diabetes.
Results: Although subjects with IDF metabolic syndrome incurred only about 56% of the total costs incurred by subjects with diabetes ($3663 vs. $6488, p<0.0001), their costs were 25% greater than subjects with neither diabetes nor metabolic syndrome ($2926, p<0.0001). Among those without diabetes, pharmaceutical costs were 45% higher when metabolic syndrome was present ($1123 vs. $776, p<0.0001), and outpatient costs were 22% greater ($1777 vs. $1461, p<0.0001). These differences remained significant even after removing the cost of treating risk factors, such as hypertension, dyslipidemia and obesity, that accompany the metabolic syndrome. Inpatient costs were nonsignificantly (11%) higher. In the multivariate model of total costs, meeting the hypertension metabolic syndrome criterion was associated with $1134 in additional costs (p<0.0001), while meeting the HDL criterion added $458 (p<0.0001). Coefficients for the other three metabolic syndrome criteria were not statistically significant.
Conclusion: Medical costs among patients with IDF metabolic syndrome are moderately elevated relative to patients without the syndrome, but do not approach the level of expense incurred by patients with diabetes. Hypertension and low HDL are the metabolic syndrome criteria most strongly associated with cost, probably because of their link to cardiovascular disease, which is expensive.



PS 73

Quality of care

0897

HbA_{1c} levels exceed recommended targets prior to initiation or intensification of insulin therapy in clinical practice: baseline data from the PREDICTIVE™ European cohort*

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Background and Aims: Although the benefits of good glycaemic control are well established, as reflected in clinical guidelines, many patients with diabetes remain sub-optimally controlled. The European cohort of PREDICTIVE™ - a multi-national, prospective, observational, study of the safety and efficacy of insulin detemir - provides the opportunity to assess glycaemic control in a large patient group.

Table 1: Physician reasons for commencing insulin detemir therapy

Reason for starting insulin detemir** (%)	Type 1 (n=7,067)	Type 2 (n=12,720)
Improve glycaemic control	75	87
Reduce plasma glucose variability	53	50
Reduce risk of hypoglycaemia	52	20
Unstable diabetes	27	17
Patient dissatisfaction with current therapy	25	27
Try new insulin	18	19
Improve weight control	15	35
Change due to insulin pen	5	4
Other	2	4
Side effects from current therapy	2	3

**more than one reason may have been stated*

Materials and Methods: The PREDICTIVE™ European cohort includes 19,911 patients with type 1 or 2 diabetes. Physician reasons (more than one could be stated) for commencing insulin detemir therapy were recorded, and baseline HbA_{1c} evaluated by pre-study therapy in patients prescribed insulin detemir for the reason to 'improve glycaemic control'. The approach to initiation or intensification with insulin detemir, as reported on the baseline CRF, was explored.

Results: The primary reason reported by physicians for commencing insulin detemir therapy was to 'improve glycaemic control' (Table 1). This subgroup of 16,423 patients, comprised 5,298 (32%) type 1, 11,042 (67%) type 2 and 83 (1%) other classified. HbA_{1c} levels according to pre-study therapy group are given in Table 2.

Of patients initiated on insulin detemir for reasons other than to 'improve glycaemic control', 58% had HbA_{1c}>7.0%. Overall, on enrolment, physicians chose to intensify insulin therapy. The proportion of patients intensified to a basal-bolus regimen, pre-study vs current therapy, were: Group 1, 1.0% vs 54.0%; Group 3, 0.0% vs 84.5%; Group 5, 0.0% vs 10.6%; and Group 6, 0.0% vs 39.6%. In Group 4 − OADs only − OADs were continued with the addition of insulin

Table 2: Baseline measures of patients initiated on insulin detemir to improve glycaemic control

	Type 1			Type 2			
	1×and 2×daily insulin	≥3 daily insulin	2×daily premix insulin	OADs only	1×bedtime insulin±OADs	2×daily insulin±OADs	>3 daily±OADs
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Number of patients (N)	702	4,444	431	3,257	2,080	1,709	5,005
Mean age, years (±SD)	38.4 (±18.3)	41.9 (±16.6)	36.3 (±19.8)	60.0 (±11.2)	60.1 (±9.9)	61.1 (±11.0)	61.1 (±10.5)
Mean diabetes duration, years (±SD)	13.5 (±11.1)	17 (±12.2)	12.4 (±11.3)	8.2 (±5.8)	12.4 (±7.0)	12.1 (±7.0)	12.9 (±7.8)
Mean BMI, kg/m^2 (±SD)	24.6 (±4.7)	25.4 (±4.5)	24.4 (±5.0)	29.5 (±5.1)	33.0 (±6.4)	30.6 (±6.0)	32.1 (±6.1)
Mean HbA_{1c}, % (±SD)	8.8 (±1.8)	8.4 (±1.6)	9.3 (±1.8)	9.0 (±1.6)	8.4 (±1.4)	8.9 (±1.6)	8.4 (±1.4)
Proportion with HbA_{1c} >7.0%	85%	84%	94%	95%	87%	94%	86%
Proportion with HbA_{1c} >8.0%	62%	54%	77%	70%	56%	69%	55%
Proportion with HbA_{1c} >9.0%	36%	27%	50%	40%	29%	38%	27%

detemir in 70% of patients (74% with a once-daily injection at bedtime or before dinner).
Conclusion: The results show that a significant number of type 1 and 2 patients are not achieving glycaemic targets, confirming a gap between guidelines and clinical reality. Insulin treatment is initiated or intensified at a mean HbA_{1c} much higher than the currently recommended targets.
*Austria, Czech Rep., Denmark, Finland, Germany, Ireland, Israel, Netherlands, Sweden, Turkey and UK
Supported by Novo Nordisk A/S

0898

Clinical and social factors affecting the quality of diabetes care in Poland - a multicenter Prospective Diabetes Care Quality Study (PDCQ Study)

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Background and Aims: Diabetes mellitus prognosis and costs are closely related to the quality of care. Improvement of medical care for 2 millions of diabetics in Poland is therefore very crucial and practical problem. As based on this assumption, the aim of the study was to assess the quality of care in patients with diabetes mellitus and to identify clinical, organizational and social factors acting as barriers for delivering high care quality.
Materials and Methods: The study was consisted with prospective, multicenter, randomized evaluation of medical, organizational and social factors in a sample of randomly selected diabetic patients from 18 centers (9 primary care clinics and 9 diabetes clinics) in 3 areas of Poland. The quality indicators were collected with standardized questionnaires for both patients and doctors. Study included 1294 adults with type 1 (17.9%) or type 2 (78.5%) diabetes. The mean age was 60.6±14.5 years with a greater proportion of females than males (54.9% vs 45.1%). The average diabetes duration was 12.1±8.6 years.
Results: The study revealed the following data: physical examination was performed in 90.7%, foot and eye examinations in 91.7% and 87.6% of patients, respectively. HbA1c was measured in 79.3%, lipids in 56.3% and microalbuminuria only in 18.1% of patients. The percentage of tested patients who were at goals in accordance to EASD recommendations was: 21.3% for HbA1C, 17.8% for glycemia, 11.1% for lipids, and 12.8% for blood pressure. The mean level of HbA1c was 7.53%. Retinopathy was present in 59.2% of patients, nephropathy in 10.7%, neuropathy in 41.4% and coronary heart diseases in 35.9%. Multiple logistic regression analysis showed that patients who had been examined at least once in the last year were above 2 times more likely to receive optimal diabetes care. Advising patients on diet, at least secondary education and earnings>760 PLN were also the predictors of high quality of diabetes care. However, older patients, with longer diabetes duration, with insulin treatment, males and living in the northern area of Poland were less likely to receive optimal diabetes care. Likelihood of optimal care in the general outpatient clinics was about 95% lower than in the specialist outpatient clinics.
Conclusion: Sex, age, diabetes duration, insulin therapy, at least one during last year physical examination, advising patients on diets, level of health care, region of Poland, education and earnings are factors affecting the quality of diabetes care. Attending primary care was associated with the worst quality of diabetes care. These results indicate for necessity of urgent improvement of diabetes care in general practice. Evidence based quality indicators included in questionnaire - a new, useful tool for monitoring and improvement of diabetes care in Polish outpatient clinics.

0899

Four years of voluntary quality management in diabetes care in Austria: effects on process quality and intermediate outcome

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Background and Aims: Forum for Quality Systems in Diabetes Care Austria (FQSD-A) is a voluntary Quality Management (QM) initiative in Austria. In this abstract, we describe the impact of 4 years of QM in FQSD-A on process and intermediate outcome.
Materials and Methods: The intervention consisted of continuous medical education workshops organized twice yearly with involvement of local opinion leaders, structured documentation of patient examinations, feedback on medical performance and continuous quality improvement in quality circles. Anonymized patient data was collected on annual examinations either in paper form or using an internet-based

application. Non-anonymized (with identifiable centers) quality of care benchmarks were sent to all participants quarterly and could be done anytime online. It was hypothesized that processes of care and the outcomes would be significantly linearly related to increased duration of participation in FQSD-A. We used multilevel logistic regression models with random center and patient intercept to compute average half year trends.

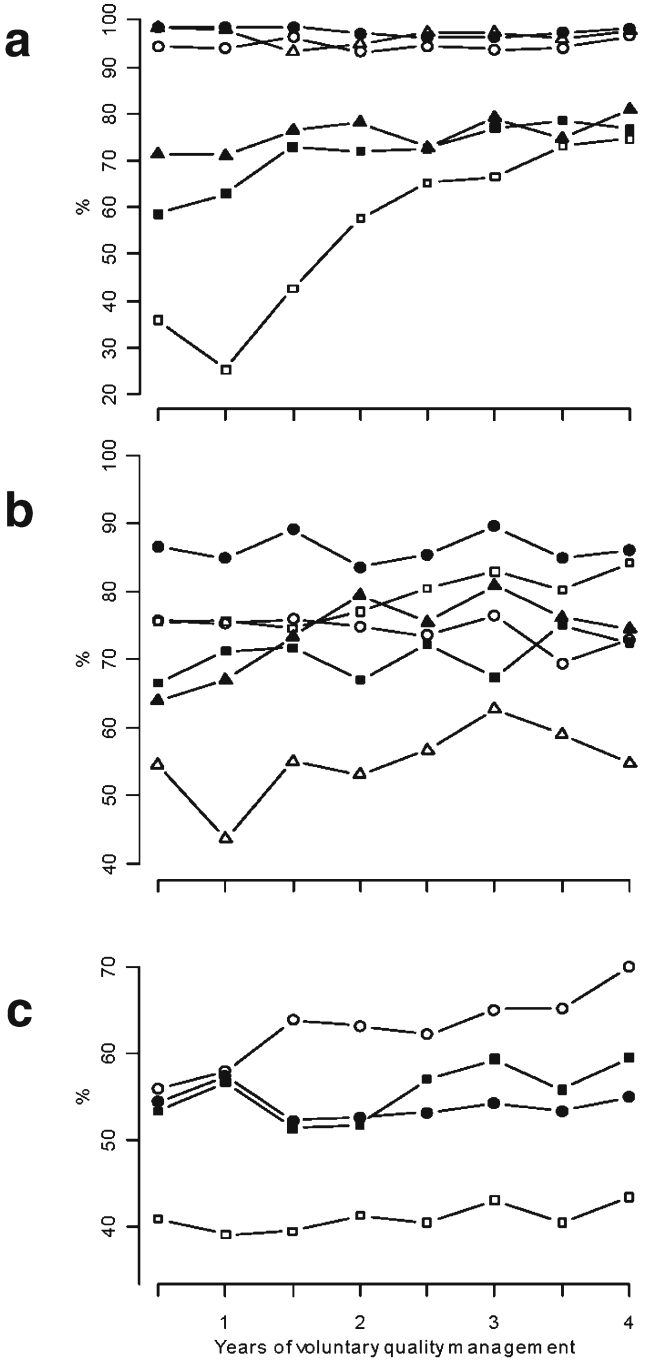
Results: In the first 4 years of continuous participation in FQSD-A, 25 health care centers collected data on 7447 diabetic patients on 9912 visits. At the first visit, patients were 61.3±15.1 years old, had an diabetes duration of 9.7±9.7 years, 56% were female and 82% had Type 2 diabetes. Processes of care and outcomes plotted against the half years of participation are given in Figure.

A - Proportion of examinations with recorded: HbA1c (p n. s.)=white circles, RR (p n.s.)=black circles, lipid profile ($p<0.001$, OR 1.96)=white squares, urinary protein or albumin excretion ($p<0.001$, OR 1.12)=black squares, smoking habits (p n.s.)=white triangles, erectile dysfunction ($p<0.001$, OR 1.16)=black triangles.

B - Proportion of examinations with: eye examinations (p n.s.)=white circles, feet examinations (p n.s.)=black circles, consecutive antihypertensive medication prescription if RR>140/90 mm Hg ($p<0.001$, OR 1.35)=white squares, prescription of antiplatelet medication in patients with CHD (p n.s.)=black squares, prescription of statins in patients with CHD and TC>200 mg/dl (p n.s.)=white triangles, proportion of patients with structured patient education ($p<0.001$, OR 1.47)=black triangles.

C - Proportion of visits with recorded: RR <140/90 mmHg ($p<0.001$, OR 1.09)=white circles, HbA1c <8% ($p<0.001$, OR 1.08)=black circles, TC <200 mg/dl ($p<0.001$, OR 1.08)=white squares and LDL <130 mg/dl ($p=0.009$, OR 1.07)=black squares.

Conclusion: Centres that continuously participated in QM for 4 years significantly improved numerous processes of care and all considered patient outcome measures. Due to the absence of control group we can not estimate the improvement attributable to QM.



0900

Pay per results. Does it improve diabetes care? A model from the UK

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Background and Aims: The new General Medical Service (nGMS) contract was implemented in the UK after April 2004. The nGMS gives financial rewards to general practitioners (GPs) for delivering enhanced clinical and organisational quality care and for improving patients' experience. Organisational standards include records and information about/for patients, whilst clinical standards include variables such as HbA1c, cholesterol levels, and blood pressure (BP) (Table).

The aim of our project is to assess the impact of the nGMS on the delivery of diabetes care in our area and whether the implementation of a pay per result system has improved the care provided to people with diabetes.

Material and Methods: We collected data from the two Primary Care Trusts (PCTs) in Shropshire, UK. Data included the number of patients with diabetes, and the numbers of patients achieving each indicator of the nGMS contract, in each practice. Shropshire covers a wide area that includes both rural and urban population. We included data from all GP practices in Shropshire (66 in total), comprising 16,858 people with diabetes.

Data were collected at four time-points: April 2004, October 2004, March 2005 and October 2005. The data for April 2004 were collected form the National Diabetes Audit (NDA). The data for the rest of the time-points were obtained from the PCTs in Shropshire.

In the analysis we have compared the mean percentage of patients achieving each indicator in each GP practice at each of the time-points.

Results: The results including means±SD, p values and 95% confidence interval for the difference in means for each of the time-points are shown in the Table.

The results show improvement in all organisational as well as the clinical standards between April 2004 and October 2005. All these improvements achieved statistical significance ($p<0.001$). Scrutiny of the data reveals that the majority of the organisational indicators improved rapidly after implementing the nGMS (October 2004), compared to April 2004, and then stabilised. Clinical indicators, i.e. control of HBA1c, BP and cholesterol, showed a sustained

Summary of indicator data at three time points. Mean(SD), 95%CI for the difference in means.

Indicator	NDA (Apr 04)	October 04	95% CI (NDA-Oct04)		P (NDA-Oct04)	October 05	95% CI (Oct04-Oct05)		P (Oct04–Oct05)	95% CI (NDA-Oct05)		P (NDA-Oct05)
BMI record	.73(.1)	.76(.11)	−.07	−.01	.004	.78(.10)	−.04	.01	.174	−.08	−.03	.000
Smoking record	.44(.14)	.89(.08)	−.48	−.41	.000	.87(.07)	−.003	.04	.103	−.47	−.39	.000
HbA1c record	.75(.15)	.85(.07)	−.14	−.06	.000	.86(.07)	−.03	−.0005	.042	−.16	−.08	.000
HbA1c <7.5	.53(.18)	.48(.12)	.01	.10	.030	.66(.09)	−.20	−.16	.000	−.17	−.08	.000
HbA1c <10.1	.90(.06)	.87(.12)	−.003	.06	.080	.94(.02)	−.10	−.04	.000	−.06	−.03	.000
Retinal screening record	.47(.21)	.58(.11)	−.17	−.06	.000	.60(.12)	−.06	.01	.113	−.19	−.08	.000
Peripheral pulses record	.22(.23)	.56(.13)	−.40	−.28	.000	.59(.12)	−.062	.004	.092	−.43	−.31	.000
Neuropathy testing record	.22(.23)	.55(.12)	−.39	−.26	.000	.59(.12)	−.07	−.01	.018	−.42	−.30	.000
BP record	.87(.06)	.91(.06)	−.05	−.02	.000	.91(.05)	−.02	.01	.274	−.06	−.03	.000
BP <=145/85	.53(.11)	.62(.10)	−.11	−.07	.000	.66(.01)	−.06	−.01	.003	−.14	−.10	.000
Microalbuminuria record	.07(.12)	.50(.20)	−.48	−.37	.000	.57(.15)	−.11	−.02	.006	−.54	−.45	.000
Serum creatinine record	.81(.09)	.83(.07)	−.043	−.002	.029	.85(.07)	−.03	.003	.105	−.06	−.02	.001
ACEi or A2 antagonist		.01(.02)				.03(.03)	−.02	−.01	.000			
Cholesterol record	.78(.08)	.80(.07)	−.04	−.004	.019	.82(.06)	−.04	−.01	.008	−.07	−.03	.000
Cholesterol <=5	.60(0.1)	.66(.07)	−.07	−.04	.000	.74(.06)	−.010	−.07	.000	−.16	−.12	.000

The data represent the mean percentage of patients who achieved the indicator in each GP practice.

improvement. For example the mean percentage of patients with HbA1c <7.5 and HbA1c <10.1 in each GP practice failed to improve initially between April 2004 and October 2004, but improved and achieved statistical significance by October 2005 (p<0.001).

Conclusions: The implementation of the nGMS contract has been associated with improvement in the care delivered to patients with diabetes, whether this care was organisational or clinical, in Shropshire. Although the more progressive improvement in clinical indicators might suggest the influence of improved practice in general, it is likely that the nGMS has had an impact. This improvement in Shropshire is likely to reflect a wider improvement in diabetes care across England.

0901

Quality of diabetes care in 35 centers in 25 countries using a European primary care clinical audit tool. *G*lobal *A*lphabet *S*trategy *I*mplementation *A*udit (The GAIA Project)

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Background and Aims: The Alphabet Strategy is a patient-centred, evidence-based clinical strategy based on the seven most important aspects of diabetes care ; ***A***dvice (diet, exercise, smoking cessation, concordance with treatment), ***B***lood pressure (<145/85 mmHg), ***C***holesterol (≤5 mmol/l), ***D***iabetes control (<7.5%), ***E***ye care and ***F***oot care (annual screen and treatment), ***G***uardian drugs, e.g., Aspirin, ACE-I. Previously it has been shown that the Alphabet Strategy has produced clinical audit outcomes comparable to UKPDS and Steno-2 intensively treated patients.

Our aim was to investigate the quality of diabetes care internationally and assess the feasibility of using the Alphabet Strategy in diabetes centres in other countries particularly outside of Europe.

Materials and Methods: Medical students from Warwick University collected data in the Alphabet Strategy format during their electives around the world in partnership with the GAIA Collaborators. The UK Primary Care Quality outcomes Framework (QoF) provides a template for clinical audit in diabetes care (maximum points 99). Audit parameters included smoking cessation advice, blood pressure and cholesterol target attainment, diabetes control, eye and feet screening, micro-albuminuria and proteinuria screening and treatment. We analysed data on 3275 patients from 35 centres, in 25 countries, in all continents bar Antarctica. The results were organised into quintiles (Q1-Q5) according to QoF points.

Results: • Overall average points 45.2±24.9, Europe 66.8±26.5 vs rest of the world 39.0±21.0 (p=0.002).

• Large variation in management of diabetes care throughout the world (QoF points range 10.2 to 87.5).

• There is a significant correlation between socio-economic parameters and quality points

• The more % GDP (Gross Domestic Product) spent on healthcare, the better diabetes care (r=0.87, p<0.01).

• The GDP correlated with quality points (r=0.80, p<0.01).

• The country's life expectancy correlated with quality points (r=0.72, p<0.01)

• Overall 57.5% of 146 healthcare professionals questioned felt that they were likely to adopt the Alphabet strategy. A similar proportion of patients were positive about the Alphabet Strategy

Conclusion: There is large variation in standards of diabetes care internationally as assessed using the UK Primary Care QoF points system. This correlated with adverse socio-economic circumstances. The Alphabet Strategy was deemed to be useful in helping to implement an evidence-based, patient education focussed strategy for delivering diabetes care. The level of support for the Alphabet Strategy was independent of economic factors and has the potential to help deliver effective diabetes care.

0902

Development of an insulin dose selection device supporting tool for visually impaired patients with insulin-treated diabetes mellitus for: FlexPen

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Background and Aims: The dose selection of insulin self-injection practice is difficult for visually impaired patients, but there is no specific insulin injection device for them. Though insulin injection device dose selection assisting device exists for InnoLet, which is contributing to QOL improvement of visually impaired patients, treatment range is narrower as InnoLet has no ultra rapid acting insulin.

Objective here is to develop an insulin injection device dose selection assisting tool attachable to FlexPen which enables dose selection by patients with visual impairment.

Materials and Methods: This is a tool with two rectangle rubber poles with a hole in each pole. An user pinches FlexPen with the rubber poles and listens to the click sound generated near the unit display section of FexPen while rotating the dial, through air type receivers connected to the poles.

Use Precision Sound Level Meter Type 2003 (Node) to measure, in anechoic room, the click sound from FlexPen from a distance of 30 cm and the sound through earphone of air type receiver when the assist tool is attached to FlexPen.
Results: Sound intensity was 28 and 29 decibels respectively.
Intensity of sound from receiver was 1.13 times higher than that at the distance of 30 cm. The clicking sound will be heard louder due to the effect of excluding ambient noise by applying receivers to both ears.
Conclusion: There has been no insulin-injecting device available for visually impaired patients needing ultra rapid acting insulin. An insulin-injection device dose selection-assisting tool for FlexPen has been developed enabling visually impaired patients to use FlexPen, and expected to contribute to the improvement of patients QOL.

0903

The efficacy of self blood glucose monitoring in type 2 diabetes: The ESMON Study

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Background and Aims: Although widely advocated by healthcare professionals there is uncertainty about the value of self-monitoring of blood glucose [SMBG] in type 2 diabetes [T2DM]. There is conflicting evidence as to whether monitoring is associated with improved diabetes control. SMBG might contribute to diabetes management by providing instant feedback to patients on glycaemic control thus positively reinforcing beneficial lifestyle habits. Conversely SMBG might have a negative effect on patient experience by increasing anxiety levels. The aim of this study was to investigate in a prospective randomised controlled trial the effect of SMBG on glycaemic control and psychological indices over a 1 year period in a cohort of patients with newly diagnosed T2DM.
Materials and Methods: Patients with newly diagnosed T2DM were randomised to a SMBG group or a non monitoring [control] group in 4 Northern Ireland Diabetes Clinics. All patients underwent a comprehensive diabetes education programme and an identical structured treatment regimen for glycaemic control based on HbA1c targets was followed in each group. Each patient was reviewed at 3 monthly intervals for 1 year. HbA1c was measured at each clinic site using a DCCT aligned HPLC assay. Patients in the SMBG group were asked to perform 4 fasting and 4 postprandial measurements per week using a single meter type provided by the investigators. Monitoring patients were instructed in the interpretation of glucose readings and advised on appropriate responses to high and low readings. Psychological wellbeing [satisfaction, locus of control, attitudes to diabetes, anxiety and depression] was evaluated by a validated questionnaire tool at each time point. The predeterminded primary endpoints reported are HbA1c and measures of psychological well being.
Results: 184 patients were randomised to the SMBG group [55 males/41 females; 57.7 (11.04) years, mean (SD)] or non monitoring [control] group [56 males/32females; 60.9 (11.5) years. There was no difference in baseline HbA1c or BMI between the groups [HbA1c 8.76(2.12)% v 8.6 (2.27)%, BMI 32(6.23)kg/m^2 v. 34 (6.98); mean (SD) monitoring v non monitoring, p=NS]. Although HbA1c levels decreased in both the monitoring and non monitoring groups there was no significant difference in HbA1c between the groups at any timepoint with 12 month HbA1c values of 6.87(0.76)% v 6.94 (1.16)%, monitoring v non monitoring, p=NS.There was no significant difference in BMI between the 2 groups at any time point with 12 month values of 31.8(6.03) v 33.1(6.32) monitoring v non monitoring, p=NS. There was a trend towards greater anxiety levels in the monitoring group when corrected for age and gender. There was no difference in other psychological indices between the groups.
Conclusions: In this cohort of patients with newly dignosed T2DM, SMBG was not associated with improved glycaemic control. There was a trend towards increased levels of anxiety in the self monitoring group.
This study was funded by the N Ireland Research and Development Office

0904

Treatment of diabetic ketoacidosis in Denmark: a national survey

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Background and Aims: The aim of the present was to examine management routines of diabetic ketoacidosis (DKA) in adult patients in departments of internal medicine in Denmark.
Materials and Methods: A questionnaire was sent to all departments of internal medicine in Denmark responsible of

managing DKA. The questionnaire requested information on management routines of DKA and a copy of the departments current management protocol.

Results: A questionnaire was sent to 71 departments of which 9 were excluded from the survey. Of the remaining 62 departments 59 returned the questionnaire and/or a copy of their management protocol, giving a response rate of 95%.

At 19 departments (32%), all patients with DKA were managed in an intensive care unit. Routines of insulin therapy varied significantly in terms of doses and routes of administration. 24 different regimens were identified.

21 different fluid protocols were identified. Isotonic saline was the preferred hydration solution by 58 departments (97%). Potassium supplements were provided as a separate infusion of either isotonic potassium-sodium-chloride (73%) or isotonic potassium-chloride (13%). Volume administered during the first eight hours of treatment could be estimated in 20 protocols (median 4750 ml, range 3750 to 7700 ml). Use of bicarbonate was endorsed by 80%.

Conclusion: This study shows significant variations in management routines of DKA in Denmark.

In many cases, the treatment routines employed are not supported by evidence from clinical trials. We recommend implementation of national and/or European guidelines for management of DKA in adult patients.

0905

Perceptions of seriousness and management of diabetes. Findings from the SHARED study (Survey comparing Healthcare professionals and patients to Assess REal perceptions of Diabetes issues)

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Background and Aims: There is growing appreciation of the shared responsibilities between patients and healthcare professionals (HCPs) in diabetes management. A better understanding of patients' views may enhance communication and clinical outcomes. SHARED is an international survey designed to investigate the perceptions and expectations of patients with diabetes, HCPs (physicians and nurses) and the general public on the seriousness and management of diabetes.

Materials and Methods: The survey was conducted between August and October 2005 and involved >12,000 individuals from 8 countries (France, Germany, Italy, Netherlands, Spain, Sweden, UK and the USA). These included the general public (n=8,024), diabetologists/endocrinologists (n=697), general practitioners (n=818), nurses (n=814) and people with Type 1 (n=404) and Type 2 diabetes (n=1,609). The survey employed the "mirror principle" (asking the same question to patients and HCPs). Participants were questioned by: telephone survey (general public [sample representative of the national population aged ≥15 years] and HCPs) or online survey (patients). Data for patients, physicians and in some cases nurses are reported.

Results: Mean age for patients was 50 years with 58% male. 46% of patients thought that diabetes was an extremely or very serious condition, while physicians anticipated that only 25% of their patients would think so. 90% of patients expressed an interest in learning more about their diabetes, while physicians believed this to be the case for only 65% of their patients. 78% of patients reported that they search for more information about their diabetes, while physicians estimated that only 56% of their patients are doing so. A majority of patients lacked knowledge about HbA_{1c}, with only 44% of patients having heard about the HbA_{1c} test. 47% of patients who were not taking insulin said they would be willing to take insulin if their physician recommended it. 62% of patients on insulin felt that their condition had improved since they had started taking insulin and 90% of those on insulin said they would be willing to adjust their own insulin doses; however, physicians and nurses thought that only 42% and 39%, respectively, of their patients would be willing to do so. In total, 91% of patients taking oral medication would like to take part in decision-making about their treatment, while physicians believed only 44% of their patients would be willing to do so.

Conclusion: Almost half of insulin-naïve patients are willing to consider insulin therapy. Contrary to the perceptions of their HCPs, those patients who are on insulin are largely positive about their treatment and are willing to self-titrate in order to improve their blood glucose levels and prevent long-term complications. There is a broad lack of knowledge among patients about HbA_{1c}.

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0906

Parameters affecting postprandial blood glucose: effects of errors - the diabetes error test model

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Background and Aims: Diagnostic and therapeutic tools for people with diabetes are characterized by rather large and varying margins of error, which increase the risk for hypo- and hyperglycemia. The Diabetes Error Test Model (DETM)

has been developed to characterize the clinical relevance of the margins of error of parameters affecting blood glucose (BG) and their relative contribution to postprandial BG.

Materials and Methods: The DETM is based on a treatment concept aimed at normoglycemia after meals by pre-prandial injections of insulin in adult people with diabetes. The model includes as parameters: a) preprandial BG measurement by patient self-monitoring (SM), b) patient estimate of carbohydrate amounts (CARB) in food, c) effect of CARB on maximum BG-increase, d) effect of insulin on maximum BG-decrease, e) insulin dosage. Covering the relevant range of pre-prandial BG (30–330 mg/dl) the DETM simulates the maximum effect of these parameters and their margins of error on postprandial BG values as outcome according to standard textbook guidelines. If the postprandial outcome is not normoglycemia but turns into hyper- or hypoglycemia, we define this as the Critical Point. If this postprandial outcome exceeds the BG-range of 50–200 mg/dl for>4.5% of all SMBG-values (sample size and range: ISO-NORM 15197:2003/E), this device is classified as unacceptable (Error Acceptance Analysis). At first, the effects of SMBG measurement errors are evaluated while all other parameters are kept at 0% error. In a second step, the combined effects of 2 parameter errors are examined as an example of daily life experience of people with diabetes.

Results: A SMBG error of+20% (classified e.g. by the Error Grid Analysis/EGA as acceptable and within the Zone A) results as postprandial outcome in normoglycemia (BG range: 60–160 mg/dl) if pre-prandial BG values are in the range of 30–130 or 260–330 mg/dl, but results unexpectedly in hypoglycemia (BG-range: 50–60 mg/dl) if pre-prandial BG values are between 131–259 mg/dl. In this range the Critical Point for hypoglycemia is already reached at a SMBG error of+11%. If the SMBG error of +20% is combined with an error of CARB estimate in the food of+20% hypoglycemia as postprandial outcome is worsened (BG-range: <50 mg/dl). In contrast, the combination of the SMBG error of+20% with an error of CARB estimate of −20% results in normoglycemia as postprandial outcome. The calculated error acceptance areas for SMBG values (Error Acceptance Analysis) are different and smaller than the combined areas of EGA-Zone A and B. SMBG devices that are still acceptable by EGA criteria (within Zone B limits) are classified already as clinically unacceptable by the Error Acceptance Analysis.

Conclusion: The DETM simulates the effects of potential errors of parameters affecting postprandial BG within the clinically relevant BG range. It analyzes the relative error contribution not only of SMBG but also of therapeutic parameters like carbohydrates and insulin per se as well as combined. The DETM evaluates the clinical relevance of these errors, distinguishes between acceptable and unacceptable parameter errors and presents a detailed risk assessment with the focus on postprandial BG outcome. No other available model for the clinical evaluation of SMBG errors has a comparable detailed analysis and hypothesis generating potential.

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PS 74

Diabetes management in outpatient care

0907

Interactive diary for diabetes: a useful and easy-to-use new telemedicine system to support the decision-making process in type 1 diabetes

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Background and Aims: A widespread use of carbohydrates (CHO) counting is limited by its complex patient educational approach. Telemedicine can represent an important tool to help patients to adjust the insulin bolus according to the amount of CHO ingested in a meal, while avoiding the use of complex calculations. The aim of this pilot study was to evaluate the applicability and acceptability of the Interactive Diary, a Bolus Calculator and a telemedicine system based on communication between patient and specialist by SMSs through mobile phones.

Materials and Methods: The Interactive Diary for Diabetes (IDD) was set up in patients' mobile phones and allowed: 1. to record the blood glucose values; 2. to quantify the total CHO assumed during a meal, by choosing from a list of pictures the specific food and the amount ingested; 3. to suggest the most appropriate bolus insulin in relation to the patient's CHO/Insulin ratio, the factor of insulin sensitivity, the blood glucose goal, and the physical activity done; 4. to send the data filed in the mobile phone as short messages (SMSs) to the physician. Four Italian Diabetes Outpatient Clinics enrolled 50 patients with type 1 diabetes, aged between 18 and 65 years, and familiar with the use of mobile phones. Socio-demographic and clinical information was collected and questionnaires investigating quality of life (SF-36 Health Survey Instrument and WHO-

DTSQ) and satisfaction with the IDD were filled in at baseline and after 12 weeks.

Results: At the end of the study, 67% of patients found the system good and 27% excellent; 66% stated the system was very or extremely helpful; 90% judged it easy to use. CHO counting and insulin bolus calculation were considered by the patients as the most useful functions of the IDD. No significant changes were documented in clinical parameters and in DTSQ-WHO and SF-36 scores after 12 weeks (table). No serious hypoglycemic episodes requiring medical intervention were reported during the study, although patients could choose food they wished, modifying their pre-meal insulin doses as suggested by the IDD.

Conclusion: This pilot study showed that the IDD was safe, easy to use and well accepted by all the patients.

Clinical data	Baseline	Follow-up	p
HbA1c	7.2±0.8	7.2±0.9	1.0
Systolic blood pressure	117.9±14.4	119.6±9.3	0.6
Diastolic blood pressure	77.4±6.7	77.6±5.1	0.6
BMI	23.4±3.2	23.6±3.4	0.8
DTSQ-WHO score	29.6±4.5	30.5±3.6	0.4
SF-36:			
Physical Functioning	87.0±14.0	90.3±10.8	0.6
Role Limitations due to Physical Problems	85.7±30.6	78.3±37.6	0.5
Bodily Pain	74.3±28.5	77.4±28.8	0.8
General Health Perception	51.9±20.0	51.1±20.7	1.0
Energy/vitality	51.4±20.4	58.1±18.5	0.4
Social Functioning	63.3±23.8	71.7±21.9	0.3
Role Limitations due to Emotional Problems	71.1±35.3	68.9±46.2	0.7
Mental Health	65.4±18.4	68.8±15.7	0.6
PHYSICAL COMPONENT SCORE	49.9±7.7	49.9±6.5	0.9
MENTAL COMPONENT SCORE	72.6±10.4	44.6±11.3	0.4

Data are expressed as means±std; * Wilcoxon test

Supported by Lifescan Inc. Milpitas, United States

0908

Internet diabetic patient management system using short messaging service automatically produced by knowledge matrix system

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Background and Aims: Several internet-based diabetic management systems have been developed, and through these systems, physicians are able to manage a lot of patients wherever internet is available. However, in previous systems, it took a lot of time for physicians to look over each patient's data, formulate an appropriate message to each patient, and send it to the patient. In our study, we developed a knowledge matrix system based on Korea Staged Diabetes Management Guideline, which is used to formulate the appropriate message through an automated algorithm, and did a clinical trial to see the effectiveness of this system in the management of diabetic patients.

Materials and Methods: We developed a knowledge matrix system for proper diet and exercise for diabetic patients based on Korea Staged Diabetes Management Guideline. Also, we invented a device that has dual function of blood glucose meter and pedometer. By simply connecting this device to the patient's cellular phone, the patient's measured data is automatically sent to his/her personal data sheet on the internet web site (http://yds.healthkorea.net). In addition, patients are to record the amount and type of food they have taken on the internet. When these data are sent to the main system, our knowledge matrix system automatically composes a message that corresponds to the patient's current status concerning diet and exercise, and this message is sent to each patient after the physician confirms this message on line. Therefore, the patient receives a regular medical recommendation through the cellular phone. And, we applied this system for 6 weeks in 32 diabetic patients who visited our hospital from January to March, 2006. Then, we compared fasting and postprandial blood glucose levels and HbA1c before and after 6 week trial.

Results: After 6 week trial of this Internet diabetic patient management system, the daily amount of exercise was significantly increased, and fasting and postprandial blood glucose levels were significantly decreased (133.87±40.97 to 121.37±26.80 mg/dL, p=0.019; 184.62±38.03 to 144.31±32.51 mg/dL, p=0.000, respectively). Moreover, HbA1c level was improved from 7.88±0.92 to 7.28±0.59% (p=0.041), and hypoglycemic event was not reported during the trial.

Conclusion: We propose this 'internet diabetic patient management using short messaging service automatically produced by knowledge matrix system' as a new tool for communication between diabetes care group and patients.

0909

Locus of control in patients with type 2 and 1 diabetes after long-term management by group care

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Background: The locus of control describes the relationship between patients' behaviour and various daily life situations. We have developed and validated a model to manage type 2 and type 1 diabetes by systemic group education (Group Care=GC) which, over 5 years, improved health conducts, knowledge of diabetes and quality of life in all patients but improved clinical variables only in type 2 patients.
Aims: to investigate the locus of control in patients with type 2 and 1 diabetes followed for at least 5 years by GC, compared with control patients followed by traditional one-to-one care.
Methods: Two questionnaires were administered to 83 patients followed by GC (56 Type 2 and 27 Type 1) and 79 controls (51 Type 2 and 28 Type 1): the Peyrot and Rubin tool, specific for diabetes, and the Wallston and Wallston, generic for chronic diseases. Both questionnaires include 18 items assessing 3 areas: Internal Control of disease, the role of Chance in changing the disease, trust in Others (family members, health operators). The mean scores in each test for the two groups and for the different diabetes patients were compared; then a multivariate model was fitted, where the score of each test represented the dependent variable and group care, type of diabetes, sex, age and duration of diabetes the independent ones.
Results: with the Peyrot and Rubin questionnaire, GC increased Internal Control independently of type of diabetes ($p<0.001$), whereas the Wallston and Wallston tool showed lower Internal Control among patients with Type 1 diabetes. Patients with Type 1 and 2 diabetes followed by GC had reduced fatalistic attitudes, as shown by both questionnaires ($p<0.001$). Both the Peyrot and Rubin ($p<0.001$) and Wallston and Wallston ($p<0.002$) questionnaires showed lower trust in Others among patients with Type 1 diabetes, which was not modified by GC. The differences of both questionnaires associated with GC were independent of sex, age and duration of diabetes.
Conclusions: Patients with type 1 diabetes may have lower Internal Control and trust in family and health operators than those with type 2. GC may increase Internal Control and reduce fatalistic attitudes without modifying trust in Others in patients with both type 2 and 1 diabetes.
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0910

Lifestyle intervention: comparison among different strategies

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Background and Aims: The ADA recommends assessment of self-management skills and knowledge of diabetes at least annually, and the provision or encouragement of continuing diabetes education.Educational techniques have shifted from educational presentations to interventions involving "empowerment" with patient participation and collaboration.The objective of this study was to compare the efficacy on glycaemic control of different educational strategies in obese adults with type 2 diabetes. We focused our behavioural intervention on weight loss and physical activity.
Materials and Methods: 152 obese T2DM patients (90M,62F), duration of disease yrs 9,5±8,5, age 63,4± 11,3 yrs, were randomly assigned to one of three intervention groups 1st group: received a short, written leaflet given by doctor. Every month a physician visits patients and gives reinforcing compliant message on life style. 2nd group: received individual dietary instructions by a dietitian. Every 15 days, a dietitian meets the patient to individualize the dietary prescription. 3rd group: received group counselling for behaviour modifications to help make healthy food choices and understanding how food affects blood glucose level. Patients learn different meal-planning approaches (pyramid and exchange list). They meet the diabetology team every two week for 4 months. Glycaemic control, lipid levels, body weight, knowledge about diabetes, medication requirements, and symptoms were monitored during the 4 months.
Results: At the end of 4 months, there were no significant reductions in total cholesterol, and low-density lipoprotein cholesterol (LDL-C) values in the 3 intervention groups. In the third group there was a significant weight loss ($p=0,01$), a significant improvement in glycosylated haemoglobin ($p=0,01$). In the third group there was a higher number of correct answers about the knowledge of diabetes.
Conclusion: The educational strategy with creation of motivation for proper diet and exercise was effective not only in weight reduction but also in better control of diabetes and in a reduction of OHA usage.

0911

Employer-based diabetes disease management: assessing the health outcome and economic benefits

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Background and Aims: Disease management initiatives aimed at improving health outcomes for diabetes patients

through enhanced self-management education and disease awareness may help reduce the growing economic burden impacting employers and health insurers. To this end, programs utilizing face-to-face, targeted, and sustained measures provided by trained caregivers with knowledge of a patient's history have been shown to reduce hyperglycemia, cholesterol and blood pressure among employee populations. The goal of this analysis was to estimate the potential for clinical benefits and cost savings through implementing a workplace diabetes wellness plan.

Materials and Methods: Clinical parameters and patient demographics were derived from results observed among a cohort of 47 patients completing a Diabetes Intensive Management Program at 9 employer groups in the US. Patients were evaluated at baseline and end-of-study for HbA1c, diabetes knowledge, medication adherence and blood glucose monitoring compliance. Those at high risk for or with confirmed diabetes were enrolled in an intensive 1-on-1 education program, with access to several interactive learning sessions. Patients' antidiabetic medication regimens were not altered during the study period: 74.5% received orals only, 6.4% insulin only, 8.5% insulin plus orals, and 10.6% did not take medication. A validated and peer-reviewed health economic model was used to project long-term clinical and cost outcomes for intensive management versus those modelled not to receive intervention based on the results of this disease management program. The model utilizes Markov structure and Monte Carlo simulation techniques to simulate the progression of diabetes-related complications. Life expectancy (LE), quality-adjusted life expectancy (QALE), incidence rates of complications and total costs were projected over a lifetime horizon. A societal perspective was adopted, with direct costs (treatment and complication) and indirect costs (workplace productivity) accounted. Costs and clinical outcomes were discounted at 3% per annum.

Results: Patients receiving intensive disease management training (mean period of 9 months) considerably improved glycemic control, LDL cholesterol and BP (absolute HbA1c: −0.5%; LDL: −33 mg/dL; BP: 140/86 to 124/83 mm/Hg). Such benefits were projected to increase LE by 0.63 years and QALE by 0.52 quality-adjusted life years compared to non-intervention. Projected cumulative incidence rates of complications were lower for those on intensive management, including 14.6% less diabetic retinopathy, 24.9% less end-stage renal disease, 10.9% less neuropathy and 9.3% less myocardial infarction events. Lifetime direct and indirect cost savings were projected to be $4,975 and $14,347 per patient, respectively, ($552 total savings per patient per year) for intensive versus non-interventional management.

Conclusion: These data illustrate the potential for clinical and economic benefits through implementing an intensive diabetes management program in the workplace. Sustained intensive training on nutritional, educational, and behavioral modification has the potential to save costs and improve patient quality of life, with benefits gained by employees, employers and health insurance groups.

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0912

Beyond good intentions: a proactive self-management intervention for patients recently diagnosed with type 2 diabetes during the ADDITION-Netherlands study

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Background and Aims: to evaluate the effectiveness of a behavioral intervention for screen-detected patients with type-2 diabetes (DM2) on top of and apart from an intensive pharmacological intervention. The theoretical background of this approach lies in the concept of proactive coping, which comprises the elements of anticipation, timing and planning of adequate behavior to deal with future threatening situations.

Materials and Methods: Patients were recruited from the Dutch ADDITION study, a screening trial which examines the effects of an intensive multi-factorial pharmacological intervention on cardiovascular risk factors in screen-detected DM2 patients. Participants were subsequently randomized to a usual care or experimental condition in which they received the course "Beyond good intentions". Both groups were followed for one year, assessing psychological outcomes (proactive coping, self-efficacy, diabetes distress and self-care behavior) and medical outcomes (weight, blood-pressure, Hba1c) at baseline (T1), at 3 months (T2) and at one year (T3). The final sample included 102 control patients and 78 experimental patients. A 2×2 factorial design was employed to evaluate the behavioral intervention (experimental versus usual care) nested within the pharmacological intervention (intensive versus usual care).

Results: Compared to controls, course participants improved significantly with regard to proactive coping, self-efficacy and self-care between T1 and T2 ($p<0.01$) and they were able to maintain these changes at T2 ($p<0.05$). Participants profited equally from the course, regardless of their medical treatment condition. The full set of medical outcomes data is expected in April 2006 but initial analyses of weight change at T2 reveals that patients in the intervention condition lost significantly more weight than controls (−2.0 versus+0.5 kg).

Conclusion: An intervention based on proactive coping is effective in helping newly diagnosed patients to manage their diabetes and it appears also to be feasible, regardless of the intensity of treatment that patients are receiving. Proactive coping theory is relevant to self-management in that it helps patients both initiate and maintain self-care behavior.
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0913

Public awareness of the importance of cardiovascular risk factors characteristic for the metabolic syndrome
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Background and Aims: The epidemic of the metabolic syndrome poses a major challenge throughout the world. Despite the current debate in the literature, the metabolic syndrome offers a simple public health concept and an easily identified starting point for clinical interventions known to reduce risk for the obesity-related type 2 diabetes and cardiovascular diseases. To prevent the metabolic syndrome, a wide social collaboration is needed and, therefore, the public awareness of the metabolic syndrome is of great importance.
Materials and Methods: To evaluate the public awareness of the metabolic syndrome, a cross-sectional survey was performed among randomly selected adult subjects aged 35 years or more in Hungary. Simple questionnaires were used by representatives of a communication bureau in 20 cities. People on the street were asked to stop for a moment to answer 14 written questions voluntarily and anonymously. A total of 1001 questionnaires (601 women, 400 men with different professions; 335 aged 35–44 years, 333 aged 45–54 years and 333 aged 55 years or more) were summarised.
Results: The term of the metabolic syndrome was known by 286 subjects (29%). In this group, subjects were informed about the syndrome from acquaintances (40%), media (29%), physicians (26%) or others (5%). In the total cohort (n=1001) clinical abnormalities were reported as follows: hypertension 323 (32%), obesity 206 (21%), diabetes mellitus 113 (11%) and lipid abnormalities 182 (18%) subjects. Forty-eight percent of the total cohort was regularly cared by general practitioners. Medical information about the metabolic syndrome provided by general practitioners were considered insufficient and superficial by more than half (52%) of the subjects questioned. The severe consequences of the clinical abnormalities were known by the majority of subjects. In a scale from 1 (neglected) to 5 (considered seriously) the mean score for hypertension was 4.19, it was 3.92 for lipid abnormalities, 4.35 for diabetes and 3.71 for obesity. As for the metabolic syndrome itself, its significance was well known (mean score 4.6 using the same scoring system). Unfortunately, 19% of subjects reported neither regular physical activity nor keeping a healthy diet.
Conclusion: In order to increase the public awareness, extensive public information campaigns should be implemented by health care providers as well as by written and electronic media focusing on the metabolic syndrome.

0914

Understanding of diabetes prevention studies: questionnaire survey of professionals in diabetes care
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Background and Aims: Framing of data is a well recognised cause of misconceptions about efficacy of health interventions. Diabetes prevention studies are reported to have shown reductions of diabetes risk by up to 60%. Since the underlying metabolic changes are small the clinical significance of this effect may be overestimated. The present survey explores the extent to which different formats of presenting study results may influence diabetes health care professionals' perceptions of the importance of intervention effects on diabetes risk.
Materials and Methods: Participants of three European diabetes conferences in autumn 2005 (160 nurse educators, 112 diabetologists or physicians with a special interest in diabetes, 27 other professionals) were presented a questionnaire that included nine items with different presentations of results of three diabetes prevention studies (Finish Diabetes Prevention Study, Diabetes Prevention Program, Women's Health Initiative). Results were presented as relative risk reductions, proportion of subjects with diabetes, fasting glucose values, HbA1c values, probability of remaining free of diabetes, and graphs with truncated scale or scale with complete range from 0.0 to 1.0. Almost identical wording was used in all items: "Looking at this result (these results, this graph), what is your impression about the effect of this (preventive) intervention on diabetes risk?" Participants were asked to mark their ratings as either "very important (++)", "important (+)", "not very important (−)" or "not important at all (−)". In addition, the UKPDS' main results on the primary endpoint were used to evaluate understanding of natural frequencies.
Results: Participation rate was 96%. Effects were interpreted as important or very important by 92% (255/276) when results were presented as proportions of subjects with

diabetes (14% intervention group, 29% control group), by 87% (248/285) when communicated as a risk reduction of 57%, by 39% (110/284) when the corresponding fasting plasma glucose values were presented (mean difference 0.3 mmol/L), and by 18% (52/283) when glycosylated haemoglobin values were used (6.0% versus 6.1%). About 73% (202/278) rated the study effect on diabetes risk as more important when the graph of the original publication with a truncated scale narrowed to the range of results (3.5% versus 4.2%) was presented rather than a graph with a scale ranging from 0.0 to 1.0. Corresponding results of the 3 diabetes prevention studies were rated as of identical importance by only 23%, 13%, and 16% of participants, respectively. Understanding of natural frequencies was poor. The intervention effect was substantially overestimated by 47% of participants. Only 2 to 6 participants answered correctly.

Conclusion: Health care professionals view the benefit of preventive interventions substantially higher when changes in diabetes risk are communicated rather than related glycaemic parameters. Transformation of continuous metabolic data into diagnostic categories may interfere with understanding of study effects.

0915

Basic characteristics and treatment regimens in people with type 1 diabetes in the international diabetes management practices study (IDMPS)

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Background and Aims: This multinational observational study is being performed to gather data that reflect current practices in the management of people with diabetes in different areas of the world and to evaluate compliance with international guidelines.

Materials and Methods: Data are being collected from type 1 diabetic patients (≥18 years) in 1-year cycles (a 2-week cross-sectional period followed by a 9-month longitudinal period) for 5 years.

Results: Data from the first cross-sectional period are available from 1327 people (51% men) in 11 countries in Eastern Europe, Asia, and Latin America. The mean age was 36±13 years, mean BMI 23±4 kg/m^2 (for both men and women) and mean disease duration 11 years. Mean HbA_{1c} was 8.2±1.96% and 27% of people had an HbA_{1c} <7%. Mean FBG (laboratory) was 161±75 mg/dL. Mean systolic/diastolic blood pressure was 122/75 mmHg. Target treatment levels were achieved for LDL (<100 mg/dL) in 41% of people, for HDL (>40 mg/dL) in 81%, and for triglycerides (<150 mg/dL) in 72% of people. A basal+prandial insulin regimen was used by 51% of people with an average daily dose of 52 IU insulin and a mean HbA_{1c} of 8.1%. Premixed insulin was used by 27% of people with an average dose of 38 IU insulin and a mean HbA_{1c} of 8.5%. Only 9% of people were using a basal insulin alone (average dose 31 IU insulin and mean HbA_{1c} 8.5%) and 4% a prandial insulin alone (average dose 29 IU insulin and mean HbA_{1c} 7.6%).

Conclusion: As can be seen, despite the findings of the DCCT and the recommendations of the ADA, the majority of people recruited have not achieved the recommended treatment goals for HbA_{1c} and FBG. It is thus apparent that more efficient guidance and education be made available to improve treatment outcomes in type 1 diabetic patients world wide.

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0916

Lipid profile modification is the most effective cardiovascular intervention in the Steno-2 Study

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Background and Aims: The majority of trials studying the impact of cardiovascular risk factor modification in diabetes care have been limited to the intervention of only one risk factor. The landmark Steno-2 Study (S2S) demonstrated the benfits of a multifactorial approach to reducing cardiovascular events and mortality in a population of patients with type 2 diabetes mellitus. We employed cardiovascular risk calculators in a mathematical model to determine the most effective risk factor intervention in the S2S.

Materials and Methods: 10-year absolute coronary artery disease risk using the Framingham based risk function (FRF) and the UKPDS Risk Engine (UKPDS) was calculated from cardiovascular data at baseline (T0) and the end of the S2S (Tfu). Instead of averaging the risk scores of individual subjects at these times, the average of each risk factor in the subgroups of male and female smokers, and male and female non-smokers were inputted into each risk estimator. Separate calculations were made for these subgroups as "average smoking" and "average sex" does not exist. To estimate the contribution made by improvement in a paricular risk factor, its average value was inputted at T0, then the exercise was repeated with its average value at Tfu, leaving the other risk variables inchanged. As ageing alone increases risk, to make a valid comparison an age-adjusted risk score was calculated by using risk variables at T0 and age at Tfu. Knowing the proportions of individuals in these subgroups allows the estimation of overall risk score improvement due to improvement in this particular risk factor. This improvement was expressed as a percentage of the total risk reduction between T0 and Tfu.

Results: Conventional arm using UKPDS - total risk reduction 38.79–33.86=4.93%. Percentage contribution of risk factors to this reduction:

- HDL increase 51.67%
- Total cholesterol rection (TC) 13.82%
- Stopping smoking 12.27%
- Systolic blood pressure reduction (SBP) 5.64%
- Glycaemic control (HbA1c) 0%

Conventional arm using FRF - total risk reduction 29.44–24.32=5.11%. Percentage contribution of risk factors to this reduction:

- HDL 61.47%%
- Stopping smoking 14.68%
- TC 12.11%
- SBP 11.74%

Intensive arm of using UKPDS - total risk reduction 36.08–21.39=14.69%. Percentage contribution of risk factors to this risk reduction:

- TC 46.51%
- HDL 27.65%
- HbA1c 10.77%
- SBP 9.95%
- Stopping smoking 5.12%

Intensive arm sing FRF - total risk reduction 27.25–15.48=11.77%. Percentage contribution of risk factors to this reduction:

- TC 45.41%
- HDL 26.06%
- SBP 22.59%
- Stopping smoking 5.94%

Conclusion: Lipid profile modification appeared to be the most effective cardiovascular intervention in both treatment arms of S2S.

PS 75

Diabetes in childhood

0917

Intensified insulin therapy in children and adolescents with type 1 diabetes mellitus - comparison of treatment with regular versus analogue insulin

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Background and Aims: Today, more than two third of all children and adolescents with intensified insulin therapy were treated with short and long acting insulin analogues. However, up to the present, in children and adolescents after starting treatment with regular and NPH insulin, the change to insulin analogues is mostly the second step. Hence, it was the aim of the trial to analyse the quality of diabetes control, the incidence of acute complications, quality of life and possible benefits of treatment with insulin analogues versus regular and NPH insulin.

Materials and Methods: Over a period of 23 months all patients (n=350) with type 1 diabetes mellitus and intensified insulin therapy admitted to our hospital were included in the trial and underwent clinical examination, analysis of self-monitoring, frequency of acute complications and an extensive test battery (including standardised analyses of diabetes self-management, handling, diabetes-related knowledge, psychological status, well-being and motivation).

Results: Mean age of patients was 12.3±3.8 ys, diabetes duration was 4.3±3.5 ys and HbA1c was 8.0±1.4%, with only 25.7% (90/350 patients) having levels≤7.0% (DCA 2000, mean normal 4.0–6.4%) (number of severe hypoglycaemia [glucose i.v., glucagons injection] 5). Out of the total group 243 patients (69.4%) were treated with insulin analogues. In comparison to patients with regular and NPH insulin, patients treated with insulin analogues were older (12.7±3.7vs11.3±3.7 ys, p=0.001), had a longer diabetes duration (4.6±3.5 vs3.7±3.4 ys, p=0.021) and a higher BMI (20.5±4.2 vs 19.2±3.8 kg/m^2, p=0.003). There were no differences between the groups in respect of HbA1c (8.0±1.1 vs 7.8±1.3, p=0.2), insulin dosage (0.8±0.3 vs 0.8.±0.3 I.U./kg bd wt, p=0.72) and the number of mild hypoglycaemia (10.8±7.0 vs 10.5±5.9, p=0.7). There was a difference between the groups regarding diabetes-related knowledge (scores: 67.0±16.3 vs 61.4±18.4 points,

Type of basal insulin	HbA1c after 4 mths [%]	HbA1c after 6 mths [%]	Mean daily BG after 4 mths [mg/dl]	Mean daily BG after 6 mths [mg/dl]	Mean BG before breakfast, after 4 mths [mg/dl]	Mean BG before breakfast, after 6 mths [mg/dl]	Total DID after 6 mths [U/kg/day]	Glargine or NPH dose after 6 mths [U/kg/day]
Glargine	7,1±0,6	7,6±0,9	153±22	161±23	148±23	143±25	0,72±0,25	0,35±0,13
NPH	7,8±0,9	7,7±0,9	160±15	165±20	155±38	168±26	0,80±0,11	0,44±0,11
P	0,03	0,76	0,47	0,66	0,65	0,03	0,34	0,07
95% CI of	0,07 to	-	-	-	-	2,37 to 46,23	-	−0,01 to 0,18

p=0.02). Analysing reasons for changing therapy from therapy with regular and NPH insulin to short and/or long-acting insulin analogues the following indications were overt (more than 100% because in some patients there was more than one indication): 1. improvement of the quality of diabetes control (71%), 2. instability of blood glucose profiles (48%), 3. Dawn-Phenomenon (10%) 3. frequent hypoglycaemia (5%).

Conclusion: At present, in Germany most of the children and adolescents with type 1 diabetes mellitus and intensified insulin admitted to hospital are under treatment with insulin analogues. There were no differences between patients treated with regular/NPH and short and/or long acting insulin analogues in respect of quality of diabetes control and the incidence of acute complications. Hence, it seems, that treatment with insulin analogues is the second step. Mostly patients changed treatment strategy due to severe problems (improvement of quality of diabetes control, instability of blood glucose profiles, Dawn-Phenomenon or/and frequent hypoglycaemia) In children and adolescents insulin analogues seems to be a very worthful tool to overcome specific problems. They should used individually.

0918

A randomized cross-over trial comparing glargine and NPH insulin in preadolescent type 1 diabetic children

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Background and Aims: Glargine is a long-acting insulin analog with flat action profile. Although it is more and more widely used as basal insulin replacement therapy, also in young diabetics, results of scrupulous trials conducted in children are sparse. The aim of this cross-over study was to compare glycemic control on Glargine and on NPH insulin (Humulin N) in type 1 preadolescent children.

Materials and Methods: 14 children (7 boys and 7 girls) aged 6–12 years (mean 10,4 years) with diabetes duration of 2–11 years (mean 6,9 years) were included into the study. The study protocol comprised for each patient (i) a 6 months' period of therapy with NPH insulin as basal insulin and (ii) a 6 months' period of therapy with Glargine as basal insulin. Patients were randomized to start either with Glargine or with NPH insulin. Patients used Humalog and/or rapid acting human insulin (Humulin R) for meal and correction boluses during all the study period. All patients measured blood glucose regularly (at least 3–4 times a day). They used the same type of memory provided blood glucose meters, that cooperated with the outpatients' clinic's computers. Results of HbA1c and 2 weeks' mean blood glucose (BG) measurements (all day and before breakfast), data concerning daily insulin doses (DID), insulin injections at 3.00–4.00 AM, weight and height as well as hypoglycemia and ketoacidosis history were recorded 4 and 6 months after starting each of two 6 month's periods of the cross-over study protocol (i.e. either on Glargine or on NPH insulin). T-test was used for statistical analysis of data.

Results: Mean values and standard deviations for main variables after 4 months and after 6 months on Glargine and on NPH respectively are presented in the Table below. Mean BMI after 6 months was 18,7 kg/m2 on Glargine and 18,5 kg/m2 on NPH (NS). Three patients on NPH insulin but none on Glargine were injected insulin at 3.00–4.00 AM. No severe hypoglycemia or ketoacidosis occurred during the study period.

Conclusion: In preadolescent children Glargine constitues an useful and safe alternative to NPH insulin providing better early morning and (at least for a short-term) good total glycemic control, not increasing the risk of severe hypoglycemia or excessive weight gain. As such, it should be reimbursed by national health services.

0919

Insulin glargine (GLAR) vs intermediate-acting insulin in adolescents with type 1 diabetes (T1DM) using multiple daily injection (MDI) therapy

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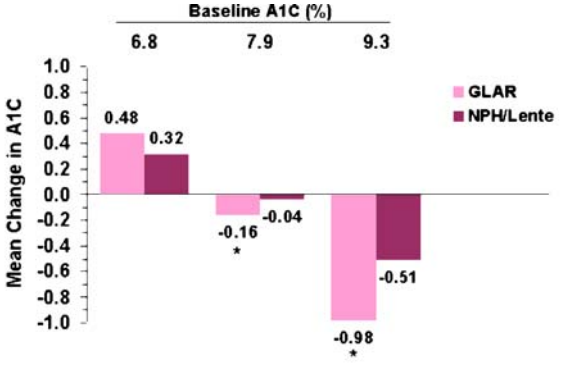
Background and Aims: The Diabetes Control and Complications Trial demonstrated that improvements in A1C reduced complications in adults and adolescents with T1DM. However, improved A1C was also associated with increased hypoglycemia. Increased variability in blood glucose (BG) may contribute to the inability to reduce A1C without increased hypoglycemia. The aim of the current study was to compare efficacy (A1C, hypoglycemia, and BG variability) of once-daily GLAR vs twice-daily intermediate-acting insulin (neutral protamine Hagedorn [NPH] or lente) as basal therapy in pediatric pts with T1DM treated with an MDI regimen including premeal rapid-acting analog (lispro).

Materials and Methods: This was a 24-wk, multicenter, randomized, open-label, parallel-group study of 175 adolescents with T1DM (GLAR, n=85; NPH/lente, n=90) who had A1C≥7.0% and≤9.5% on any insulin regimen. Basal doses began at ~40–50% of total daily dose and were titrated to target fasting BG (≥70 and≤100 mg/dL). In a subset of pts (n=90), BG variability was assessed using the Continuous Glucose Monitoring System (Medtronic MiniMed®). Measures included SDs of BG values and measures of glycemic excursion, such as MAGE (mean amplitude of glycemic excursion) and M value (?/N absval[10log {SMBG/120}]^3+[{Max-MinSMBG/20}]).

Results: Treatment groups had similar demographics: mean age of 13 years; diabetes duration 5 years; body mass index 23 kg/m^2; A1C 7.9%; 82% of pts were white. There was a small reduction in mean A1C from baseline to endpoint in both groups (GLAR=−0.18%; NPH/lente=−0.03%). However, there was a significant interaction between treatment effect and baseline A1C; GLAR pts with high baseline A1C (mean A1C of ≥7.9%) had greater A1C reduction than NPH/lente pts (Fig.). Mean daily basal insulin dose at 24 wks was significantly greater (*P*=.0064) for NPH/lente (50.7±2.1 U) vs GLAR (42.4±2.1 U). Mean total daily insulin dose was similar between groups (*P*=NS).



*P<.05, between groups. Baseline values represent modeled effect of baseline A1C

Number of pts with confirmed clinically relevant hypoglycemia (BG <36 mg/dL) [GLAR 28 vs NPH/lente 30] and severe hypoglycemic events (GLAR 9 vs NPH/lente 4) and possibly related adverse events (AEs) was comparable between groups (*P*=NS). BG variability was significantly less for GLAR vs NPH/lente at 24 wks as demonstrated by SD and MAGE (SD, −13.4 vs −2.4, *P*=.0147; MAGE, −34.4 vs −2.5, *P*=.0055). The M value also tended to be lower with GLAR vs NPH/lente (*P*=.0631).

Conclusion: These modeled effects show that in adolescents with high baseline A1C values, treatment with GLAR results in greater A1C improvement compared with NPH/lente despite significantly lower doses of GLAR and comparable rates of hypoglycemia and AEs. Pts treated with GLAR also have less variability in BG.

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0920

The impact of insulin regimens and dose adjustment on glycaemic control in 21 centres

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Objective: To evaluate the impact of different insulin regimens and insulin adjustment strategies on metabolic control in a large international cohort of adolescents with type 1 diabetes.

Methodology: Questionnaires were completed by adolescents and their parents/carers attending sequentially in 21 international centres. Questionnaires investigated age, gen-

der, diabetes duration, insulin regimens, insulin adjustments and glycaemic targets. HbA1c (DCCT adjusted) was measured centrally.

Results: Questionnaires were completed by 2062 adolescents (age 14.4 y+/−2.3 y, male 50.6%, diabetes duration 6.1 y±3.5).

Different insulin regimens were used both within and between the 21 different centres. Mean HbA1c=8.2% SD 1.4, with significant influence of age ($r=0.1; p<0.001$), gender (females 8.3%±1.5, males 8.1%±1.3; $t=3.0$; $p<0.005$), diabetes duration ($r=0.29; p<0.001$). Insulin regimens used and analysed were pre-mixed insulin twice daily (7%), free mixing insulin twice daily (7%), basal bolus (39%), pump treatment (15%). For these four groups there are significant differences between groups ($F=13.9$; $df=3$; $p<0.001$). Individuals free mixing had significantly better glycaemic control than all other groups (mean HbA1c 7.6%; SD 1.3). There was no difference between pump users (HbA1c 8.1%; SD 1.3) and individuals on basal bolus regimens (HbA1c 8.3% ; SD 1.5) with both these groups having significantly lower HbA1c than individuals on BD pre-mix regimens (HbA1c 8.6% ; SD 1.6). Free mixing was mainly used in two centres and within those centres no difference was observed between the free mixing individuals and the remaining group.

Adolescents stating that they adjust insulin therapy based on blood sugar levels ($F=4.4; p<0.004$) and their intended food intake had significantly lower HbA1c levels than those who do not adjust ($F=6.0; p<0.001$) as did those who give extra doses for additional snacks ($F=3.01; p<0.05$). Also centres with lower HbA1c had more individuals making insulin adjustments. Young people who test their blood glucose when managing hypos have better HbA1c than those who eat until they feel better ($F=3.9$; $p<0.01$). Both the target for ideal HbA1c perceived by adolescents and the result they would be happy with in clinic were associated with the actual HbA1c achieved (ideal $r=0.24$; happy $r=0.41$).

Conclusion: Insulin regimens per se have less impact on metabolic control than adjustment strategies and perceived targets. Further analysis of data in this study will look at which other factors including education are most influential in diabetes management and outcome.

Supported by Novo Nordisk

0921

Quality of care of children and adolescents with type 1 diabetes mellitus in new EU accessing countries (the DEPAC Study)

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Background and Aims: According to an IDF-Europe initiative, a baseline evaluation of diabetes care of 11085 diabetic patients has been performed in the Czech Republic, Estonia, Hungary, Latvia, Lithuania, Poland, Slovakia and Slovenia in 2005 (the DEPAC Study). We assessed separately the data of children and adolescents with Type 1 diabetes mellitus.

Materials and Methods: Data of 281 patients, <18 yrs of age with Type 1 diabetes mellitus (boys:48.45%, girls 51.55%) were analyzed (mean age boys: 13.04 yrs, girls: 12.70 yrs). The mean time from diagnosis was 5.27 yrs. Patients were randomly enrolled. Questionnaires for each patient were completed by a diabetologist. Questions included data about age, sex, duration of diabetes, type of treatment, metabolic control (HbA1c), lipid data (total cholesterol, LDL cholesterol, triglycerides and HDL cholesterol), blood pressure (BP) and data about short and long term complications. Data were analyzed centrally in the University of Warsaw.

Results: The mean HbA1c was 8.78%, and the percentage of patients with an HbA1c <7.0% was 16.66%. Self monitoring of blood glucose was available in 93.47% of patients. Human insulin was used for treatment in 67% and analogue insulin in 57%.Two third of patients were treated on the basal-bolus regimen, and 28% of them with an insulin pump (CSII). Targets for total cholesterol levels (1.1 mmol/L) in 86.57%-; targets for LDL cholesterol (<2.5 mmol/L) in 62.79%-; and targets for triglyceride levels (<1.7 mmol/L) were achieved in 90.50% of patients. The target for systolic BP (<120 mm Hg) was achieved in 97.12%-, and for diastolic BP (<80 mmHg) in 98.52% of patients. The prevalence of severe hypoglycaemia - within the last six months- was 4.47% ; and of ketoacidotic coma was 2.41%.We found that the prevalence of non-proliferative diabetic retinopathy to be 2%, and of microalbuminuria to be 4%. The prevalence of diabetic neuropathy was 5%. Unexpectedly, peripheral arterial disease was reported in two boys, and other forms of CHD was found in two girls. The percentage of smokers among adolescents was 9.97%. No significant differences between boys and girls has been found except in the mean HDL cholesterol level and in the percentage of smokers ($p<0.02$ and $p<0.05$, respectively).

Conclusions: Despite a good access to insulin and diabetes supplies, the average level of metabolic control and of serum lipids is far from desirable in young diabetics. The results should be analyzed in national health services for the options of improved care and education of patients.
Supported by Novo Nordisk

	T1DM males	*Control males*	*P*	*T1DM females*	*Control females*	*P*
Tobacco	47.1%	38.1%	<0.05	54.5%	55.1%	NS
Cannabis	33%	37%	NS	25%	28%	NS
Alcohol	56%	61%	NS	65%	51%	<0.05
Other drugs	2%	3%	NS	5%	3%	<0.01

0922

Adolescents with type 1 diabetes and risky behaviours

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Background and Aims: A growing body of literature indicates that adolescents with chronic conditions are as likely, or more likely, to take risky behaviours than their healthy peers. Very few data exists about adolescents with type 1 diabetes mellitus (T1DM). The aim of our study was to assess whether adolescents with T1DM in Northern Italy differ from their healthy peers in risky taking behaviours.
Materials and Methods: Data were drawn from 187 patients attending 4 summer camps, aged 12–20 yrs (mean 14±2 yrs) and with a mean disease duration of 7±5 yrs (range 1–19 yrs). During the camp each of them filled an anonimous confidential questionnaire to determine the prevalence of sexual behaviour, drug use (tobacco, alcohol, cannabis, cocaine, and synthetic drugs), and only in T1DM group the frequency of mismanagement related to diabetes care. The control group comprised 464 healthy adolescents recruited among high school students. Personal, family and school related variables were analysed to ensure comparatibility between groups. Analysis was carried out separately by gender. Chi-square, Fisher's and Student's tests were used to compare categorical and continuous variables.
Results: The T1DM group showed similar rates of sexual intercourse among males and lower rates among females (34.1% vs 35.5%, p NS, and 27.3% vs 41.4%, p<0.05, respectively). For most studied drugs, T1DM males reported higher rates of use than control group only about tobacco, while females showed similar or higher rates for every drug studied (see table). Among T1DM group, the patients who engaged in risky behaviours showed higher rates in tratment mismanagement (76% vs 34%, p<0.01).
Conclusion: Adoelscents with T1DM in our sample are as likely, or more likely, to take risky behaviours than their healthy peers, and should receive the same anticipatory guidance, also because this could lead to a worsening of glycemic control.

0923

Factors of low grade inflammation in type 1 diabetes children and adolescents

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Background and Aims: Fibrinogen and C-reactive protein are the markers of inflammation and its slightly elevated plasma level in subject with type 2 diabetes indicates a risk for cardiovascular events. Pathogenesis of subclinical inflammation in type 1 diabetes it has still been poorly investigated.
The aim of study was to determine the CRP and fibrinogen plasma level in metabolically stable, free from microangiopathy type 1 young diabetics and analysis of significant factors low grade inflammation.
Materials and Methods: We analyzed 190 type 1 diabetes (92 female/98 male), mean age 11.54 SD 4.13 y; mean diabetes duration 4.47 SD 3.96 y (0,1–17,6) mean $HbA1_c$8.46 SD 2.18% (4.9–19). During schedules visits clinical and biochemical parameters: CRP, Fibrinogen, $HbA1_c$, Total-, LDL-, HDL-cholesterol, trigliceride, C-peptide, antibody TPO, TG, IgA_{EmA}, IA_2, GAD were assessed. After examinations subjects with severe hypoglycaemia, DKA (ketoacidosis), infections or endocrine diseases were excluded.
Results: 17% of study population had plasma fibrinogen level above age-related range, this group presents significantly higher C-reactive protein (0.5 mg/dl vs 0.167 respectively p=0.019), but metabolic control, BMI, diabetes duration and age was comparable to group with normal fibrinogen range. After adjustment for age, diabetes duration, gender, BMI, total-, LDL-, HDL -cholesterol, positive fibrinogen correlation with antibody TPO (r=0.22 p=0.0045), TG (r=0.12 p=0.0022) was found. Additional analyzes in group with increased risk of microvascular complications (HbA1c>8,5% and diabetes duration>5 years) did not revel correlation with fibrinogen and CRP plasma concentrations.
Conclusion: This preliminary analysis indicates that low grade inflammation parameters correlate with non metabolic and diabetes related factors in children with type 1

diabetes. Autoimmune thyroid process is a significant factor involved into this pathology.

0924

3-month safety and efficacy of an oral insulin (Oral-lyn™) administered at lunchtime in juvenile type-1 DM subjects maintained on basal glargine insulin and pre-breakfast and pre-dinner regular insulin

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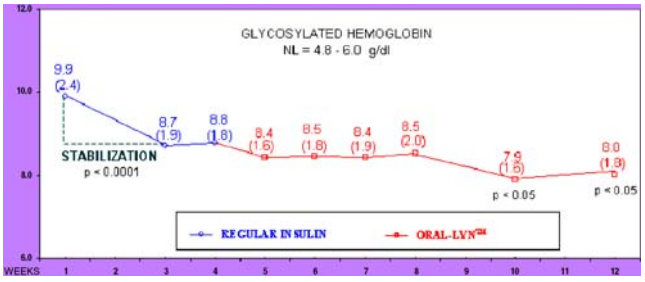
Background and Aims: Participating subjects in this 6-month phase are 24 adolescents (12M; 12F) and 5 young adults (2M; 3F) referred to us in various degrees of metabolic control as reflected by altered baseline measurements of glucose 236.6 (116.6) mg/dL; fructosamine 472.6 (126.6) umol/L; and glycosylated hemoglobin (HbA1c) 9.8 (2.3) g/dL. Baseline demographics of the 24 adolescents are: Age 14.7 y (2.1); Bone age 14.1 (2.2); CA/BA 1.0 (0.1); Height 153.8 cm (9.4); Weight 51.0 kg (10.2); BMI 21.7 (3.2); DM since onset 6.7 (2.8). Baseline demographics of the 5 young adults are: Age 20.6 y (2.2); Bone age (BA) 18.8 (0.4); CA/BA 1.1 (0.1); Height 161.1 cm (12.8); Weight 62.5 kg (9.3); BMI 23.0 (1.8); DM since onset 7.0 (1.7). Mean age for the entire group is 15.7 y (3.0); BA 14.9 (2.7); CA/BA 1.1 (0.1); DM since onset is 6.8 (2.6).
Materials and Methods: After a stabilization phase using basal s.c. glargine insulin and 3 pre-prandial s.c. regular insulin injections, standard therapy continued for 2 additional weeks for comparison. Immediately thereafter, split doses of Oral-lyn™ immediately before and after lunch replaced the lunch-time injection of regular insulin. Patient-collected glucose values in the initial phase, fructosamine and HbA1c were compared weekly. Values are reported at the end of each phase.
Results: Results are shown in the chart and graph.
Conclusion: Fructosamine and HbA1c results at the 12th week of treatment demonstrated a trend similar to that obtained with standard therapy. In summary, successful replacement of regular s.c insulin for Oral-lyn™ at lunchtime during the initial 3 months of this 6-month trial was achieved.



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Gestational diabetes

0925

Insulin secretion is markedly reduced in women with previous gestational diabetes

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Background and Aims: Gestational diabetes (GDM) constitutes a risk factor for progressive glucose metabolism deterioration throughout the post-pregnancy years. To explain this aspect, the hypothesis tested by this study was that a previous GDM modifies insulin sensitivity or insulin secretion even in women who remain glucose-normotolerant after the preganancy.
Materials and Methods: Gestational diabetes (GDM) constitutes a risk factor for progressive glucose metabolism deterioration throughout the post-pregnancy years. To explain this aspect, the hypothesis tested by this study was that a previous GDM modifies insulin sensitivity or

	FRUCTOSAMINE	FRUCTOSAMINE	HbA1C	HbA1C	GLUCOSE (BASAL)	GLUCOSE (BASAL)
PHASE	MEAN	SD	MEAN	SD	MEAN	SD
BASELINE	476.89	130.22	9.9	2.4	**236.6**	116.5
REGULAR INSULIN (end of week 6)	368.2	91	8.4	1.6	140.4	35.5
ORAL-LYN (end of week 9)	379.1	133	8.5	2.1	143.3	39.9
COMPARED TO BASELINE		**<0.0001**	**<0.0001**	**<0.0005**		
ORAL-LYN (end of week 12)	390.6	98.9	8.0	1.8		
COMPARED TO REG. INSULIN		**NS**	**<0.005**			

insulin secretion even in women who remain glucose-normotolerant after the preganancy.

Results: In spite of similar mean values of BMI, age, time from pregnancy and glucose-area-under-curve, ISIogtt (mg/dl per min±SD) was significantly lower (3.75±1.73) in the IGT group than in those with GDM (4.91±2.45) and with NGT (5.25±2.71; $p<0.05$, after ANOVA), while the median value (range) of insulin secretion (mU/mmol,) was greatly decreased in GDM group: 18.6 (35–45) compared to IGT-group: 33.2 (9–109) or NGT: 26 (10–86); $p<0.05$ after ANOVA. Median disposition index (range) was consequently markedly lower in GDM-group: 76 (15–460) than in IGT-group: 98 (27–423) or in NGT-group:130 (31–465). These differences remained unchanged after adjusting for age, BMI and post-partum lag-time.

Conclusion: Even in presence of a normal glucose tolerance, the women with previous GDM present a modest reduction in insulin sensitivity and a marked decrease in insulin secretion, and consequently of the disposition index, as compared to those who had normal or impaired glucose tolerance during a previous pregnancy.

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0926

First and second trimester insulin resistance and subsequent development of pregnancy induced hypertension

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Background and Aims: The association of insulin resistance and pregnancy induced hypertension (PIH) is now widely accepted but their causal relationship is not yet established. Prospective studies are necessary to establish this relationship; these are relatively scarce and still inconclusive. Also technical and ethical issues in measuring insulin sensitivity in pregnancy are major obstacles in conducting studies in this area. The present study was undertaken to investigate insulin sensitivity in first and second trimesters of pregnancy simultaneously by homeostasis model assessment and plasma sex hormone binding globulin (SHBG) and to explore the causal relationship of insulin resistance on the subsequent development of PIH.

Materials and Methods: A total of 216 pregnant women participated in the study up to their end of pregnancy. Among them 24 women developed PIH. By a nested case control design, 53 age- and BMI-matched normotensive pregnant women as control were randomly selected from the Non-PIH subjects. PIH was diagnosed by standard criteria; proteinuria was measured by urinary protein-creatinine ratio. Plasma Insulin level was measured by Microparticle Enzyme Immunoassay (MEIA). Insulin secretory capacity (%HOMA B) and insulin sensitivity (%HOMA S) were assessed by Homeostasis Model Assessment (HOMA). Plasma SHBG (Sex Hormone Binding Globulin) was measured by Chemiluminescent Immunometric Assay.

Results: The two study groups were age-and BMI-matched [age, median (range); in years: 25(22–32) in Control and 25 (20–34) in PIH; BMI, median (range); kg/m^2: 21.78(15.22–27.89) in control and 21.71(16.44–32.05) in PIH groups. There was no significant difference in fasting insulin level between the two groups [median (range); μU/ml: 5.7(1.5–70) in control and 6.1(1.4–29.8) in PIH, $P<0.71$]; SHBG [median (range); nmol/l: 133.0 (17.3–180) in control and 139.5(10.5–180) in PIH, $P<0.72$]; and HOMA S [median (range) 146.9(12.4–442.5) in control and 136.8(28.1–294.6) in PIH, $P<0.74$]. %HOMA B showed significantly higher value in PIH group compared to control. [median (range) 111.2(36.2–325.9) in control and 141.4(49–381.6) in PIH, $P<0.05$]. When the data was analyzed separately in 1st and 2nd trimesters of pregnancy, there was no significant difference between the two groups in any trimester. Using 25–75th percentile of SHBG of the control the reference range of SHBG was 84.5–180 nmol/l. Among the PIH cases 6.5% had SHBG values below lower cut -off point and 24.7% of the PIH cases had values above the lower cut- off point (Odds ratio 1.11; 95% CI, 0.34,3.60).

Conclusion: The present data demonstrates that early pregnancy insulin resistance, with HOMA S or SHBG as markers, may not be predictive for development of PIH in later stages of pregnancy.

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0927

Resistin levels are associated with insulin sensitivity in pregnant women with and without gestational diabetes

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Background and Aims: Resistin, which has been proposed to play a role in obesity-mediated insulin resistance and diabetes, is expressed in placental tissue. It is known that resistin levels increase in the second half of normal

pregnancy, although the relevance and physiological role that it could play in the pathogenesis of the insulin resistance observed remains unknown. To study the relationship between resistin and adiponectin levels and insulin sensitivity in pregnant women with and without gestational diabetes mellitus (GDM).

Materials and Methods: Fifty-eight pregnant women, 23 with GDM and 35 with normal pregnancy (NP) were studied. At the time of the OGTT (26–30 weeks of gestation), anthropometric and clinical characteristics were recorded. Baseline resistin and adiponectin levels were determined. Glucose and insulin levels were measured at baseline and 60′, 120′ and 180′ postload. Insulin sensitivity was calculated using the ISIogtt (1). Statistical Analysis: Independent samples t-test, chi-square test and multiple regression analysis.

Results: There were no differences between both groups in age, week of gestation at the time of the OGTT, pre-pregnancy and current BMI, neonatal birth weight and the week of delivery. Resistin levels were significantly lower in GDM women than in NP group (4,7±2,29 vs 9,62±2,43 ng/ml, respectively; p<0.001), but adiponectin levels were similar in both groups (11,62±3,46 vs12,40±5,04 μg/L). Insulin sensitivity was significantly higher in the NP group than in the GDM group (9,97±4,17 vs 3,23±1,07, respectively; p<0,001). The ISIogtt index correlated with resistin levels (r: 0.587; p<0,001) but a negative correlation was found between Insulin sensitivity and pre-pregnancy BMI (r: −0,364; p<0,05) and current BMI (r: −0.414; p<0.001). When a multiple linear regression analysis was developed including ISIogtt as dependent variable and age, current BMI, adiponectin and resistin as independent variables, all the variables except age were independently associated with ISI ogtt (r^2: 0.633, p<0.001).

Conclusion: Resistin levels are lower in GDM women compared with women with a Healthy Pregnancy, and resistin levels are independently associated with insulin sensitivity in pregnancy.

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0928

Serum concentrations of retinol binding protein 4 in women with gestational diabetes

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Background and Aims: Gestational diabetes mellitus (GDM) is characterized by various features of the metabolic syndrome. Different mechanisms leading to insulin resistance in GDM have been discussed. Recently, the adipocyte derived retinol binding protein 4 (RBP4) has been shown to cause insulin resistance. However, clinical data on RBP4 are limited. We hypothesized that RBP4 is elevated in women with GDM who are in a temporary state of insulin resistance.

Materials and Methods: According to a cross sectional study design RBP4 serum concentrations were measured in 48 women with GDM (mean age: 32 yrs.) and in 56 pregnant controls (mean age: 29 yrs.). An oral glucose tolerance test and anthropometric measurements were performed in all subjects. Insulin resistance was estimated by Homeostasis Model Assessment (HOMA-IR).

Results: RBP4 serum concentrations were comparable between women with GDM and controls (median: 32.2 [interquartile range: 17.3–50.5] μg/ml vs. 28.0 [IQR: 13.6–48.9] μg/ml; P=0.382). Women with GDM had higher body mass index (BMI), HOMA-IR, insulin concentrations and fasting as well as post load blood glucose levels compared to controls (all P<0.05). In a correlation analysis RBP4 was associated with BMI (R=0.29; P=0.047) and fasting glucose (R=0.33; P=0.024) but not with other clinical parameters in subjects with GDM. In contrast, RBP4 only correlated with insulin (R=0.37; P=0.005) and HOMA-IR (R=0.37; P=0.006) in controls.

Conclusion: The adipokine RBP4 was neither elevated nor related to insulin resistance in women with GDM. Therefore RBP4 is unlikely to play a role for insulin resistance in patients with GDM.

0929

Plasma taurine in women with history of gestational diabetes

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Background and Aims: Taurine is the most abundant amino acid in the human body and seems to play an important role in increasing glucose-mediated insulin secretion, as well as in programming beta-cell maturation during the prenatal life in utero. Aim of this study was therefore to test the hypothesis that plasma taurine is related to glucose tolerance, insulin sensitivity and insulin secre-

tion in subjects with history of β-cell dysfunction such as women with history of gestational diabetes (GDM).
Materials and Methods: We studied 72 non-diabetic women with history of GDM (n=43), impaired glucose tolerance (IGT; n=7), and normal glucose tolerance (NGT; n=22) as previously classified by a 100 g-3 hr-OGTT performed between the 24th and the 28th gestational week. Insulin sensitivity (ISIogtt, calculated through Matsuda-DeFronzo index) and a *proxy* for insulin secretion (basal plasma C-peptide/fasting plasma glucose; CP/FPG) were measured during and after pregnancy. Plasma taurine was measured after a median period of 6 yr (2–11 yr) from the index pregnancy, when glucose tolerance was retested by a 75 g-2 hr-OGTT.
Results: Plasma taurine (μmol/l±SD) was significantly lower in women who had experienced GDM (88.4±30.5) as compared to IGT (110.1±34.2) and NGT (119.8±52.1), and was unrelated to ISIogtt. Moreover plasma taurine was inversely related to previous gestational area-under-curve of glucose and directly related to post-gestational CP/FPG, as well to CP/FPG measured during the pregnancy ($p<0.05$ for both). The relative risk of altered glucose metabolism during previous pregnancies (IGT+GDM) was higher as plasma taurine decreased, even after adjusting for age, time-lag from pregnancy, body mass index and family history of diabetes (OR: 0.980;CI95%:0.963–0.999, p=0.003).
Conclusion: Plasma taurine seems to be a fair predictor of altered glucose metabolism during past pregnancies in women with antecedent GDM and appears to be inversely related to the previous as well as to the actual insulin secretion in these subjects.
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0930

Comparison of high sensitive CRP levels and serum selenium concentrations during gestational diabetic and uncomplicated pregnancy and after delivery: a follow-up study

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Background and Aims: There is increasing evidence on the importance of oxidative stress in gestational diabetic pregnancy. The essential trace element selenium, apart from its pivotal functions in several antioxidant enzymes, has also insulin-mimic properties *in vitro* and *in vivo*. In a previous study a highly significant inverse correlation ($p<0.01$) was observed between inflammatory biomarker hsCRP values and serum selenium concentrations in both gestational diabetic and uncomplicated pregnancy. The aim of our present study was to compare hsCRP levels and serum selenium concentrations during pregnancy and 8–10 months after delivery. The glycaemic status of previously gestational diabetic women was also re-assessed.
Materials and Methods: The hsCRP concentrations and serum selenium levels of gestational diabetic pregnant women (n: 20), control pregnant women (n: 20) and non-pregnant controls (n: 20) were compared. Blood was taken between the 24th and 28th week of pregnancy when the oral glucose tolerance test was performed according to WHO guidelines. The oral glucose tolerance test was repeated in former gestational diabetic study participants 8–10 months after delivery. HsCRP was measured by immunturbidimetry. Selenium was determined by hydride generation atomic absorption spectrometry.
Results: HsCRP values of both gestational diabetics (8.9±6.0 mg/l) and control pregnant women (6.3±5.8 mg/l) were significantly higher than in controls (2.7±3.7 mg/l). After delivery, hsCRP values returned to normal in both former gestational diabetics and control pregnant women (2.4±2.2 and 4.6±5.8 mg/l, respectively). The serum selenium concentrations of gestational diabetics (52.0±10.7 μg/l) and non-diabetic pregnant women (40.5±8.0 μg/l) were significantly lower than in non-pregnant controls (77.4±14.8 μg/l). Cholesterol, HDL, LDL and triglyceride concentrations were significantly higher in pregnant women compared to controls, with no difference between the two pregnant subgroups. Differences in serum selenium and lipid concentrations disappeared after delivery. When the oral glucose tolerance test was repeated 8–10 months after delivery, 14% of former gestational diabetics were diagnosed with IFG and 14% with IGT. After delivery, serum selenium concentrations no longer correlated significantly with hsCRP values.
Conclusion: The higher hsCRP in gestational diabetic and control pregnant women may indicate increased subclinical inflammation and lipid peroxidation during pregnancy. The highly significant inverse correlation between lower selenium concentrations and hsCRP values in pregnancy disappears after delivery, when serum selenium levels spontaneously increase and hsCRP values decrease. The marked decrease in serum selenium observed during pregnancy may be attributed to an increased requirement for this element in pregnancy.

0931

C-reactive protein (CRP) and insulin resistance in gestational diabetes mellitus (GDM)

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Background and Aims: To investigate the changes and the effects of CRP in GDM.
Materials and Methods: We investigated serum sCRP in 135 GDM, and the correlation with their gestational weeks, body mass index (BMI), blood pressure(BP), heart beating rate(HBR), serum insulin levels and glucose levels during 75 g OGTT, their serum lipids and urine albumin/creatinine (Alb/Cr).
Results: Ninety nine GDM had normal sCRP levels (group 1, G1) which were 1.34±0.83 mmol/l and 36 GDM had increased sCRP levels (group 2, G2) which were 5.73±3.14 mmol/l. There was no difference of gestational weeks between the two groups. Body weight of both pre-pregnancy and pregnancy were significantly higher in G2 as well as their BMI respectively. Significant higher glucose levels of 0 (G2 5.38±1.87 vs G1 4.49±0.77 mmol/l, p=0.041) and 120 min (G2 9.07±1.87 vs G1 7.86±1.21 mmol/l, p=0.012), HbA1c (G2 5.79±0.58 vs G1 5.30±0.57%, p=) as well as 30 min (G2 132.61±44.95 vs G1 70.96±37.97 mmol/l, p=0.020) and 120 min (G2 110.59±43.34 vs G1 68.25±37.71 uIU/ml, p=0.049) insulin levels during OGTT were found in G2. TG (G2 3.13±1.22 vs G1 2.39±1.36 mmol/l, p=0.047) and HBR (G2 110.19±13.34 vs G1 86.72±14.23/min, p=0.000) was also higher in G2. But no differences of Alb/Cr and BP between the two groups were found. Significant positive correlations of sCRP with gestational week, pre-pregnant and pregnant body weight and BMI, HBR and insulin injection doses. No correlation was found between maternal CRP and infant birth weight.
Conclusion: Body weight and BMI of both pre-pregnant and pregnant may play an important role in the sCRP increasing in GDM. GDM with increased CRP may have higher glucose, TG and insulin levels. They look like having severe insulin resistance.

0932

Circulating concentrations of asymmetric dimethylarginine and endothelial function in women with gestational diabetes

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Background and Aims: An increase of circulating asymmetric dimethylarginine (ADMA), an endogenous inhibitor of the nitric oxide synthase, has been reported in women with previous gestational diabetes (GDM) and other patients at risk of endothelial dysfunction and atherosclerosis, but has never been studied in the course of GDM. Therefore, the aim of our study was to compare ADMA level and other markers of endothelial function between subjects with GDM and healthy pregnant women.
Materials and Methods: The group studied consisted of 56 patients with GDM, 68 pregnant women with normal glucose tolerance (NGT) and 36 healthy non-pregnant women. Plasma concentrations of ADMA, endothelin-1 (ET-1), soluble vascular cell adhesion molekule-1 (sVCAM-1) and sE-selectin were measured by enzyme - linked immunoassay (ELISA).
Results: ADMA concentrations were significantly lower in pregnant women with NTG (68.2±21.2 ng/ml) in comparison to GDM subjects (75.7±33.7 ng/ml) and healthy controls (76.5±21.6 ng/ml, p<0.005). In contrast, ET-1 concentrations were comparable between GDM (0.79±0.29 pg/ml) and NGT pregnant women (0.79±0.34 pg/ml), and markedly higher than in non-pregnant subjects (0.62±0.18 pg/ml, p<0.01). There were not significant differences in sVCAM-1 and sE-selectin levels between the groups studied. Endothelin-1 levels correlated slightly with fasting plasma glucose (r=0.163, p=0.04) and glycaemia 60′ after glucose load (r=0.215, p=0.016). In a multiple regression analysis ADMA concentrations were associated with gestational week (beta=0.384, p=0.019) and glycaemia 120′ after glucose load (beta=0.505, p=0.008), whereas ET-1 levels were associated with gestational week (beta=0.480, p=0.015) and insulin level 120′ after glucose load (beta=−0.457, p=0.038).
Conclusion: The results suggest that in pregnancy maternal vascular function is modulated, at least in part, by a reduction of ADMA - the mechanism, which seems to be altered in GDM subjects, and that ET-1 may play a role in maintaining peripheral vascular tone in pregnancy, both - normal and complicated by GDM.

0933

Peripheral monocyte visfatin gene expression is increased in normal and gestational diabetes pregnancy

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Background and Aims: Visfatin is a newly identified adipokine, highly expressed in human visceral fat. Visfatin’s plasma levels are increased with expanding obesity as well as in type 2 diabetes, and it is thought to

promote accumulation of visceral fat, most propably acting through the insulin receptor pathway. We examined whether visfatin/PBEF mRNA production by peripheral monocytes is altered in normal and in gestational diabetes (GDM) pregnancy and whether this might relate to monocyte TNFα expression and to indices of insulin resistance.

Materials and Methods: We studied 18 women with normal pregnancy (Gestation), 15 with gestational diabetes (GDM), and 26 non-pregnant controls, all premenopausal. All pregnant women were studied during the 3rd trimester. We developed a real-time quantitative RT-PCR using fluorecently labelled hybridization probes to detect relative visfatin/PBEF and TNFα mRNA levels in isolated blood monocyte-enriched mononuclear cells (Light Cycler, Roche). Visfatin/PBEF and TNFα mRNA levels were calculated as the relative ratio of the fluorescence acquisition of visfatin or TNFα mRNA divided by that of β-actin, using the Relative Quantification Software (Roche). We also measured fasting and 2 hour post-OGTT plasma glucose and insulin (RIA) levels.

Results: Visfatin/PBEF mRNA levels were significantly higher in peripheral monocytes from pregnant (normal and GDM) women compared to controls (Table) and correlated with monocyte TNFα mRNA ($r=0.478$, $p<0.001$), which was also higher in pregnancy. We did not observe any correlation of visfatin/PBEF mRNA levels with indices of insulin resistance overall or among pregnant women. Visfatin/PBEF mRNA expression was comparable between normal pregnant and gestational diabetic women.

	BMI	Insulin fasting	HOMA-IR	Visfatin/ PBEF	TNFα
	kgr/m^2	mU/L		mRNA	mRNA
Control	27.3±1.3	9.3±1.2	2.1±0.3	0.6±0.2	0.03±0.006
Gestation	26.9±1.4	11.0±1.3	2.2±0.3	1.8±0.2*	0.06±0.02*
GDM	25.7±1.0	12.6±1.3*	2.9±0.9	2.3±0.8*	0.09±0.04

*, $p<0.05$ vs control

Conclusion: Peripheral mononuclear visfatin/PBEF and TNFα mRNA production is elevated in normal and gestational diabetes pregnancy. This might contribute to the insulin resistance that characterizes these conditions and might increase their atherogenic risk.

0934

Gestational diabetes is negatively associated with HLA DQ6

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Background and Aims: The aim of this study was to investigate the association between HLA genotypes and gestational diabetes, as well as to compare the distribution of HLA genotypes and islet autoantibodies in mothers with type 1 diabetes (T1D) and gestational diabetes (GDM) and their offspring.

Materials and Methods: HLA genotypes were determined in dried blood spots of cord blood taken from 32,663 children born to non-diabetic mothers and 1179 children born to diabetic mothers in DiPiS (Diabetes Prediction in Skåne) study. In addition, 1046 diabetic mothers were HLA typed. Out of them, 208 reported having type 1 diabetes and 838 had gestational diabetes. GAD65, IA-2 and insulin autoantibodies were determined both in cord blood and in mothers' plasma taken at delivery in standard radioligand binding assays. GDM mothers positive for islet autoantibodies were excluded from the HLA distribution analysis.

Results: 1) As expected, T1D was strongly associated with the high risk HLA DQB1 genotypes and the association was passed on to the offspring ($p<0.0001$ for DQB1*0201/0302, 0302/X and 0302/0604). There was no positive association between HLA and GDM. 2) The HLA DQB1*0602 allele, that is considered protective for type 1 diabetes, was confirmed to be negatively associated with T1D ($p=0.04$ for DQB1*0602–3–4/X), but was also negatively associated with GDM ($p=0.01$ for DQB1*0602/02, $p=0.04$ for DQB1*0602/0301 and $p=0.0008$ for DQB1*0602/X), as was also the case for the offspring ($p=0.0008$ for DQB1*0602/02 and $p=0.02$ for DQB1*0602/X) 3) A positive association was found between maternal age and GDM ($p<0.0001$) 4) T1D and GDM mothers gave birth prematurely (<37th week) in a higher frequency than the control population ($p<0.0001$)
5) Approximately 61% and 6% of children born to T1D and GDM mothers respectively, were antibody positive in cord blood. No significant association was found between islet cell autoantibodies and high risk HLA alleles in both mothers and offspring.

Conclusion: Maternal age is a risk factor for GDM. High risk HLA genotypes for T1D are not associated with GDM. The HLA DQB1*0602 allele is negatively associated with GDM and may protect from the disease.

Association between T1D, GDM and HLA DQB1 high risk genotypes in DiPiS mothers and children

		0201/0302	0302/X	0302/0604
T1D mothers	**p value**	<0.0001	<0.0001	<0.0001
	OR	12.2	4.5	9.1
	95%CI	[8.3–18]	[3–6.7]	[5–16.6]
Offspring	**p value**	0.002	<0.0001	<0.0001
	OR	2.3	2.3	5.1
	95%CI	[1.4–4.1]	[1.6–3.5]	[2.7–9.5]
GDM mothers	**p value**	0.8	0.9	0.8
	OR	1	1	1.1
	95%CI	[0.7–1.5]	[0.8–1.3]	[0.6–1.9]
Offspring	**p value**	0.2	0.8	0.6
	OR	1.2	0.9	1.2
	95%CI	[0.9–1.7]	[0.7–1.2]	[0.7–2.1]

Association between T1D, GDM and HLA DQB1 genotypes protective for T1D in DiPiS mothers and children

		0602–3–4/X	0602/02	0602/0301	0602/X
T1D mothers	**p value**	0.04	0.3	none	0.3
	OR	0.3	0.5		0.6
	95%CI	[0.1–0.9]	[0.2–1.5]		[0.2–1.4]
Offspring	**p value**	0.01	0.3	0.8	0.05
	OR	0.3	1.3	0.8	0.4
	95%CI	[0.1–0.7]	[0.7–2.3]	[0.4–1.7]	[0.2–0.9]
GDM mothers	**p value**	0.4	0.01	0.04	0.0008
	OR	0.9	0.6	0.6	0.5
	95%CI	[0.6–1.1]	[0.4–0.9]	[0.4–0.9]	[0.4–0.7]
Offspring	**P value**	0.07	0.0008	0.9	0.02
	OR	0.7	0.5	1	0.7
	95%CI	[0.6–1]	[0.3–0.7]	[0.7–1.3]	[0.5–0.9]

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Type 1 diabetes in pregnancy

0935

Respective contributions of pregnancy planning in type 1 diabetic women and insulin treatment regimen to glucose control and fetal malformations

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Background and Aims: Beside planning of pregnancy in type 1 diabetic women, the effectiveness of insulin treatment regimen is a potential determinant of fetal malformations. Our study assessed their respective relationships with glucose control during pregnancy and occurrence of fetal anomalies.
Materials and Methods: We analyzed 107 consecutive pregnancies in type 1 diabetic women followed in our clinic between 1994 and 2004. 59 (55%) had been planned. Insulin therapy at conception time and during pregnancy included multiple daily injections of regular and NPH human insulins (group I, n=35), or lispro and NPH insulins (II, n=11), insulin pump infusing human regular insulin (III, n=32) or infusing lispro insulin (IV, n=29). Rates of pregnancy planning were 26 (I), 54 (II), 75 (III) et 69 (IV), respectively.
Results: Mean HbA1c (%) at conception time and at the end of each quarter was significantly different between groups (p<0.0001): 8.66/8.08/6.98/7.06 (I), 7.67/6.62/6.31/6.43 (II), 7.44/7.08/6.68/6.72 (III), 7.46/7.10/6.65/6.82 (IV). Numbers of fetal malformations were 9 (28%), 1 (9%), 2 (7%) and 0 in groups I, II, III and IV, respectively. Pregnancy planning was independently associated with HbA1c levels (p<0.0001), whereas pump use or insulin type were not (p=0.07 et 0.16). However, insulin pump use was independently associated with a minor risk of malformations (OR 0.116 [0.024–0.561], p=0.0074), as well as use of lispro insulin but with a lower significance (p=0.046 by univariate analysis).
Conclusion: Our data confirm the benefits of pregnancy planning on glucose control at conception time and during pregnancy in type 1 diabetic patients, while use of insulin pump by itself appears as a mean to reduce dramatically the risk of fetal malformations. Use of insulin lispro shows no deleterious outcome. Including insulin pump use in pregnancy planning looks as an option to be promoted to improve pregnancy outcomes in type 1 diabetic women.

0936

Results of pre-pregnancy care in diabetic women

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Background and Aims: Prepregnancy clinics (PPC) in women with pregestational diabetes (DM) aim to improve perinatal outcome; a meta-analysis has established that they are associated to better HbA1c at the beginning of pregnancy and lower rates of congenital malformations (CM).
The aim of this study was to analyze the results of a PPC.
Materials and Methods: We studied the characteristics and outcome of diabetic patients attending the PPC initiated in March 1986 and pregnant women with DM without prepregnancy care delivering in the same period. Chisquare

tests were used to compare distributions and non-parametric tests to compare quantitative variables. Significance was set at p<0,05.

Results: In the PPC, 282 women (92.9% Type 1 DM), have been attended; 64.5% achieved a condition deemed adequate for pregnancy and 75.5% got pregnant. Median follow-up lasted 40 weeks and significant modifications were observed in HbA1c (− 0.713%) and body mass index (+0.215 kg/m2). Four hundred and sixty-eight pregnancies (86% Type 1 DM) have been attended. Pregnant women with and without PPC attendance were different in: rate of Type 1 DM (92.9 vs 78%), age (30 vs 29 years), DM duration (11 vs 9 years), gestational age at initiation of gestational care (1 vs 9 weeks), first HbA1c (6.0 vs 6.8%), smoking habit (36 vs 52%) and caesarean section (64.3 vs 53.8%); they did not differ in DM severity (White classification), initial body mass index, miscarriages, final HbA1c, hypertension, preterm delivery, major CM (5.2 vs 7.9%), minor CM (5.8 vs 9.1%), macrosomia and perinatal mortality.

Conclusion: In women with DM and acceptable metabolic control, PPC attendance is associated with better intermediate indicators but differences in perinatal outcome do not reach signficance.

0937

Audit of an integrated care pathway for pregnant women with diabetes; improved delivery of care and outcome in a UK hospital

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Background and Aims: A national audit of diabetes and obstetric care for women with pre-gestational diabetes (CEMACH- Confidential Enquiry into Maternal and Child Health) was carried out in the UK between 2002 and 2003. Key Findings from the CEMACH report (2005):

1.Women with diabetes are poorly prepared for pregnancy
2.All mothers should be entering pregnancy with substantially better glycaemic control
3.Women with diabetes are at greater risk of experiencing preterm delivery and caesarean section
4.Women with diabetes gave birth to larger babies
5.One third of term babies were admitted to a neonatal unit; 10% in general population

As a participating centre in this audit, we realised that our own documentation could be improved and the Integrated Care Pathway (ICP) for the Management of Diabetic Women during pregnancy was developed.

Materials and Methods: Between 2000 and 2003, 55 pre-gestational diabetic pregnancies were identified. An audit was performed comparing care; first before and then after introduction of the ICP, and second against the national data.

Results:

Comparison of Pre-ICP and ICP Pregnancies to the CEMACH Report

Key Finding	CEMACH	Pre-ICP: n/total (%)	ICP: n/total (%)
Total no. of pregnancies	3808	25	30
Early loss (<24w)	326 (8%)	4	0
Perinatal death rate [CI]	32 [23–40]	40	33
% pre-pregnancy counselling	35%	5/25(20%)	9/30 (30%)
% HbA1c within 6 m of conception	37%	22/25(88%)	21/30(70%)
% HbA1c measured by 13w	72%	19/21(92%)	N=26(100%)
% HbA1c <7% by 13 w	38%	3/21(14%)	9/26(34%)
% preterm delivery rate	36%	10/21(48%)	11/30(37%)
% caesarean section	67%	14/21(67%)	26/30(90%)
% babies born>4000 g	21%	5/20 (25%)	8/28 (28%)
% term admissions to neonatal unit	33%	16/20(80%)	26/28(93%)
Average stay (days)	3	12	3

Conclusion: The results demonstrate that there remains a significant deficit in the care of women with diabetes, both nationally and locally, such that the St Vincent Declaration will remain unmet in the near future. This audit demonstrates specifically where our service differs from the national standards and where we need to invest greater efforts through education and/or resources. The introduction of the ICP as a single, comprehensive record of care for all health care professionals has improved the standards of care for these women. Whilst there is a much higher caesarean section rate in the ICP group, there was a shift from emergency to elective procedures following the introduction of the ICP (elective caesarean sections as a proportion of total caesareans 30% vs 8% following introduction of the ICP). Transfer to neonatal unit policy reflect local services at this time. This audit is a stimulus to improve care for women with diabetes in pregnancy.

0938

Effect of glycaemia control initiated at different stages of pregnancy on complications and pregnancy outcomes

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Background and Aims: Glycemic targets, achieved by appropriate treatment of type 1 diabetes (T1DM), should mimic those of normal pregnancies, and metabolic state should be such that the fetus does not recognize its mother as diabetic. Our aim was to assess the effect of glycemia control, that was initiated at different stages of pregnancy, on complications and pregnancy outcomes.
Materials and Methods: In total, 118 women with T1DM were enrolled in the study (mean age−26±7 yrs; diabetes duration − 10.2±5.4 yrs); they were separated into 3 groups (Gr.): Gr.1–38 patients (pts), who received preconception care (PC), Gr.2–36 pts enrolled in the study at <10 weeks of gestations, Gr.3–44 pts enrolled in the study at>10 weeks of gestation. Pts with nephropathy were excluded. Strict metabolic control and fetal surveillance were performed throughout the pregnancy.
Results: We obtained following HbA1c levels(%): before PC − 8.7±2.36(Gr.1); 1st trimester(tr.)−6.23±0.99(Gr.1), 9.41±2.62 (Gr.2), (P=0.000); 2nd tr.- these values dropped to 5.78±1.02 (Gr.1) and 6.47±1.09 (Gr.2), and were 8.07±2.28 for Gr.3, being statistically higher, than in Gr.1 and 2 (P=0.000, P=0.001); 3rd tr. − 5.18±0.64 (Gr.1), 6.62±1.04 (Gr.2), 6.58±1.12(Gr.3) (P=0.000 - Gr.1–2; P=0.000 - Gr.1–3). Microalbuminuria had 2 (5.2%) - Gr.1, 5(13.9%) - Gr.2, and 7(20.45%) - Gr.3 pts. In the 3rd tr. their number increased to 6(15.8%), 9(25%), and 12 (20.45%) respectively. Preproliferative retinopathy was found in 9.09% -Gr.1, 33.3% -Gr.2 and in 29.4% -Gr.3 pts, proliferative retinopathy was found in 18.1% -Gr.1, 25.9% - Gr.2 and in 20.5% -Gr.3 pts. There was no retinopathy progression after delivery in Gr.1 and 2, while in two (4.5%) Gr.3 pts retinopathy progression was observed. In Gr.1, 2 and 3 following pathologies were observed: pre-eclampsia 13.5%, 19.4% and 47.7%; eclampsia − 0%, 8.3% and 13.3%; preterm delivery − 2.6%, 11.1% and 20.4%; macrosomia − 18.4%, 13.8% and 15.9% respectively. Mean infants' birth weight (g) was 3 562±568 (Gr.1); 3 473±518 (Gr2), and 3 349±637 (Gr.3); Cesarean section was performed in 59.09%- Gr.1, 77.7% - Gr.2 and 61.7% - Gr.3 pts; perinatal deaths were registered in 2 (5.5%) - Gr.1, and 6 (13.6%) - Gr.3 pts; spontaneous abortions had 2 (5.2%) - Gr.1, and 4 (11.1%) - Gr.2 pts; fetus malformations were registered in 2 (4.5%) - Gr.3 pts.
Conclusion: Good glycemia control achieved prior to and during pregnancy not always prevents macrosomia, though significantly reduces the risk of spontaneous abortions, fetus malformations and perinatal deaths, prevents pre-eclampsia and eclampsia. When preconception care is carried out, pre-eclampsia and preterm delivery are less possible, than when supervision is initiated after conception. No retinopathy progression is observed, when women are well-controlled throughout the pregnancy.

0939

Type 1 diabetic pregnant women may develop postpartum thyroditis indepently of the presence of anti-thyroid antibodies

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Background and Aims: Postpartum thyroiditis (PPTD), which is a disorder of autoimmune nature, occurs with increased frequency in women with type 1 diabetes mellitus (DM1). No conclusive data exist for gestational diabetes mellitus (GDM). In a previous study we showed that the prevalence of PPTD in DM1 women was 73% higher compared to controls, and that GDM was not associated with increased prevalence of PPTD. The aim of this study is to evaluate the presence of anti-thyroid antibodies (anti-TPO and anti-TG), during pregnancy and after delivery, as a risk factor for the appearance of the PPTD in normal, GDM and DM1 pregnant women.
Materials and Methods: We prospectively examined 148 pregnant women attending our outpatients over a period of 4 years during pregnancy, as well as during the first postpartum year. All women were of Greek ethnic origin. PPTD was defined as the *de novo* appearance of TSH levels outside the normal range 0.2–4.36 μU/ml. Women with pre-existing autoimmune thyroid disease, or taking thyroxin were not included. 44 of them were normal, (group A), 55 presented with GDM (group B), and 49 with DM1 (group C).
Results: The distribution of the anti-TPO abs before and after delivery in the three groups was as follows: group A 13.6% vs. 43.2% ($p<0.01$), group B 27.3% vs. 40% ($p<0.05$), while for group C was 36.7% vs. 42.9% (NS) respectively. Regarding the anti-TG abs the distribution was: group A 15.9% vs. 56.8% ($p<0.01$), group B 14.5% vs. 54.5% ($p<0.01$), while for group C it was 49.0% vs. 44.9 (NS). PPTD was found in 13.6% of group A, 10.9% of group B, and 24.6% of group C. From among the women with positive ant-TPO abs during pregnancy, PPDT was found in 50% of group A, 20% of group B, and 39% of group C, while from among those with negative anti-TPO abs, PPDT was found in 8% of group A, 7.5% of group B, and 16.1% of group C. With respect to the presence of positive anti-TG abs during pregnancy, PPDT was found in

28.6% of group A, 0% of group B, and 37.5% of group C, while of those with negative anti-TG abs, PPDT was found in 10.8% of group A, 12.8% of group B, and 12% of group C. Taking groups A and B together, the presence of DM1 gave an OR 2.9 for the development of PPDT in pregnant women with negative anti-TPO abs and an OR 6.8 in those with positive anti-TPO abs. Respectively, the presence of DM1 in pregnant women with negative anti-TG abs gave an OR 7.5 for the development of PPDT and 24.6 in those with positive anti-TG abs.

Conclusion: DM1 pregnant women appeared with higher anti-thyroid antibodies during pregnancy, compared to normal and GDM women. However, after delivery, in the DM1 group there was no further increase in anti-thyroid antibodies, in contrast to the other two groups (GDM and controls) in which a significant increase in both anti-TPO and anti-TG abs was observed. Finally, DM1 pregnant women present a higher risk for the development of PPDT compared to normal and GDM women, regardless of the presence or not of anti-thyroid antibodies.

0940

Concentration of sE-selectine and sVCAM-1 in early pregnancy as predictors of fetal growth in pregnancy complicated with pre-gestational diabetes

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Background and Aims: Pregestational diabetes (PGDM) is known factor influencing fetal growth. Pregnancy related hyperglycemia induces excessive fetal growth, whereas impaired function of placental vasculature result in intruterine fetal restriction. Macrosomia defined as birth weight exceeding 4000 g complicates up to 40% of all diabetic pregnancies and diagnostics of this complication still needs an improvement.The aim of this study was to check if altered concentration of markers of diabetic endothelial dysfunction can be of any use in prediction of fetal growth disturbances in diabetic pregnancy.

Materials and Methods: 97 pregnant women with PGDM and singleton pregnancy were enrolled into the study. Average age in the study group was: 26,5±5,5 years, average age at diabetes onset: 17±8,5 years, average diabetes duration: 9,5±6,5 years, average HbA1C concentration at booking: 7,3±1,8%.In the study group venous blood was taken twice: at booking and in term pregnancy. Following parameters were assessed: sE-selectine, sVCAM-1, HbA1c, lipid profile, as well as average daily glycemia and 24-h creatinine clearance.After delivery all patients were retrospectively allocated to LGA, SGA,or AGA group according to birth weight of newborn. Statystical analysis of data was pwerformed using Kruskal-Wallis test and Wilcoxon test, as well as ROC curve analysic, p value <0,05 was considered statistically significant.

Results: No significant differences in HbA1C, average daily glucemia and lipid parameters both in early and in term pregnancy were found comparing AGA, LGA and SGA group. Significant differences between groups were noted, comparing early pregnancy concentrations of sE-selectine and sVCAM-1: 39,2 (8,6–160,4) ng/ml, 53,1 (12,5–139,1) ng/ml, 39,0 (9,9–64,8) ng/ml (for sE-selectine in AGA, LGA and SGA group, respectively) and 576,8 (249,9–1666,3) ng/ml, 524,1 (224,7–1931,9) ng/ml, 846,2 (361,3–1846,0) ng/ml (for sVCAM-1 in AGA, LGA and SGA group, respectively). In the ROC curve analysis following markers of endothelial dysfunction were predictors for macrosomia: sE-selectine concentration in early pregnancy (AUC=0,67, cut-off value=42,2 ng/ml, sensitivity=38,7%, specificity=60,7%), sVCAM-1 concentration in early pregnancy (AUC=0,63, cut-off value=348,4 ng/ml, sensitivity=34,4%, specificity=85,5%), change in sVCAM-1 concentration during pregnancy (AUC=0,35, cut-off value=605,9 ng/ml, sensitivity=31%, specificity=61%).

Conclusion: 1/Measurements of sE-selectine and sVCAM-1 concentrations in early pregnancy can be useful in prediction of fetal growth abnormalities in pregnant women with PGDM.

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0941

Platelet disaggregation and intracellular levels cGMP and cAMP in pregnant women with gestational diabetes mellitus and type 1 diabetes mellitus

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Background and Aims: The diabetes mellitus (DM) of any type already at early stages is characterized by abnormality of platelets. At the patients with DM the rising intravascular activation of platelets resulting in to occurrence in a vascular channel platelet aggregates and dysfunction of microcirculation is observed. It is known, that volume of NO synthesis in platelet at the patients with Type 1 and Type 2 DM make no more than halves from recorded at the healthy persons. However now is shown, that at the pregnant women with gestational diabetes mellitus (GDM) the activity NO-synthase is much higher, than in group of the healthy pregnant women. We have shown that the partial decay of stable platelet aggregates (disaggregation)

can be induced by activity of some exogenous substances increases concentration of cGMP and cAMP in the platelets of the healthy donors. The aim of this study was to measure the velocity of platelet disaggregation and the concentration of the platelet second messengers cGMP and cAMP by sodium nitroprusside (SNP) and ATP, respectively, *in vitro* in pregnant women with GDM.

Materials and Methods: We compared 14 pregnant women with GDM (24.68±5.12 years and gestational age (24–26 weeks)), 11 pregnant women with Type 1 DM (24.31±3.68 years) and 20 healthy pregnant women (23.96±5.18 years). There were no significant differences in fasting glycaemia (5.38±0.41) mmol/l for GDM, (6.27±0.21) mmol/for pregnant women with Type 1 DM and (4.61±0.17) mmol/l for healthy pregnant women) or in glycated hemoglobin levels (6.78±0.21%, 6.94±0.13% and 5.83±0.61% accordingly). The study was performed in accordance with the principles of the Declaration of Helsinki as revised in 1996. Platelet disaggregation was determined by light transmission using an AP 2110 computerized analyzer of platelet aggregation (SOLAR, Belarus). Concentrations of cAMP and cGMP were determined radioimmunologically with a γ-counter (LKB Wallac, Finland).

Results: The nonreversible aggregation was induced by 1.5 μmol/l ADP, thus the intracellular concentration cGMP in each group has made 0.4±0.1 pmol. The donor NO - NP in concentration 400 μmol/l added to aggregating under activity ADP to platelets for 3 minutes of aggregation, in this case level cGMP was increaseds in 4 times in the first and second groups and in 5.5 times in the third group of the donors. However differences between groups were inappreciable (1.6±0.1 pmol, 1.5±0.1 pmol and 2.2±0.1 pmol accordingly). Thus the velocity of induced disaggregation at the pregnant women with GDM and with Type 1 DM was higher than in control group in 1.8 and 3.4 times accordingly. There was no significant difference between ATP-induced disaggregation and cAMP levels in pregnant women with GDM, pregnant women with Type 1 DM and healthy pregnant women.

Conclusions: Is assumed that the NP-induced platelet disaggregation in investigation groups of the donors is not linked to cGMP-signaling systems and under activity of ADP are formed less stable platelet aggregates, than in control group. ATP-cAMP pathways is not activated in investigation group.

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Diagnosis and treatment of diabetes in pregnancy

0942

Performance of the 1 hour glucose value as a diagnostic test for gestational diabetes (World Health Organization Procedure)

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Background and Aims: Diagnostic criteria for gestational diabetes are based on glucose tolerance tests performed in 2 or 3 hours. The objective of the study is the evaluation of the ability of the 1 hour glycemia after a 75 g glucose oral load to predict gestational diabetes (WHO criteria) and selected pregnancy outcomes.

Materials and Methods: A cohort of 4998 pregnant women with 20 years or more, no previous diabetes, who attended prenatal care clinics in Brazil answered a questionnaire and had a 2 h glucose test between their 24th and 28th gestational weeks.

Results: For the diagnosis of gestational diabetes the area under the ROC curve of the 1 h glucose was 0,903 (0,886–0,919). The cut point that jointly maximized sensitivity (83%) and specificity (83%) for detection of gestational diabetes was 141 mg/dl; for macrosomia detection it had sensitivity of 33% and specificity of 78%. Specificity of 99% for gestational diabetes and of 97% for macrosomia was reached with values of 180 mg/dl. An intermediate cut point with sensitivity of 62% and specificity of 94% in the prediction of gestational diabetes was 160 mg/dl; for macrosomia its specificity was 90%. The prediction of adverse gestational outcomes was similar to that reached with the 2 h glucose.

Conclusion: It is possible to simplify the detection of gestational diabetes with the 1 h glucose, simultaneously as a screening (141 mg/dl) and as a diagnostic (180 mg/dl) test. A small proportion of women will require confirmation, but the diagnosis will have been made earlier for those with higher glucose values.

0943

New threshold for impaired fasting glucose: impact on postpartum reevaluation of glucose metabolism disorders in women with gestational diabetes mellitus

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Background and Aims: In 2003 the American Diabetes Association (ADA) decreased the cut-off value of fasting plasma glucose (FPG) for the diagnosis of impaired fasting glucose (IFG) to ≥5,6 mmol/l. The aim was to increase the sensitivity in the diagnosis of glucose metabolism disorders and the agreement between abnormal categories according to FPG and plasma glucose after an oral glucose tolerance test (OGTT). Our aim was to measure the impact of the adoption of new criteria (WHO1998→ADA2003) in the postpartum reevaluation of women with gestational diabetes mellitus (GDM). We measured the influence on the prevalence of glucose metabolism disturbances and the agreement between fasting and post-OGTT categories.
Material and Methods: 1168 women with GDM were reevaluated with an OGTT within 18 months after delivery. 55% had required insulin treatment during pregnancy. At evaluation, age was 33.4 years (18.1–45.6) and body mass index was 24.28 kg/m^2 (16.6–50.17). OGTT was performed 4.8 months (0.12–17.9) after delivery. The distribution of glucose tolerance according to the different criteria was compared with a χ^2 test and the agreement between fasting and post-OGTT categories with the *kappa* index.
Results: The prevalence of glucose metabolism disorders was 16.9% according to WHO1998 criteria vs 28.8% according to ADA2003 criteria. (70% increase; $p<0.01$). The agreement between fasting and post-OGTT categories was bad with both criteria (*kappa* index 0.247 with OMS1998 vs 0.200 with ADA2003).
Conclusions: In women with GDM, the reduction in the threshold for IFG diagnosis increases substantially the prevalence of glucose metabolism disorders at the postpartum reevaluation. There is a bad agreement between basal and post-OGTT categories with both criteria, suggesting a different physiopathology.

0944

Standard breakfast validity in the diagnosis of gestational diabetes

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Background and Aims: The diagnosed of Gestational diabetes(GD) is based on an oral glucose tolerance test (OGTT). This test commonly induces vomiting, nausea or unspecific unpleasant state and is recognized as a not very physiological test. Our aims is to analyse the criterion validity of a standard breakfast for the Gestational Diabetes diagnosis in pregnant women, using as gold standard the100 g oral glucose tolerance test and National Diabetes Data Group criteria.
Material and Methods: Design: Multicenter observational study about diagnostic test in Primary Care. Measurements: A SB (711 Kcal, 98 g carbohydrates, 29 g of fat, 15 g of proteins and 3 g of fibber) in pregnant women was carried out in 24–28 gestation weeks, determining venous plasma glycaemia (VPG) in 0, 1st, 2nd and 3rd hours. We analyse sensibility(Se), specificity (Sp), positive and negative predictive values (PPV and NPV). We use the ROC curve to study the diagnostic performance and to seek the best cutoff values.
Results: 637 pregnant women accepted the study: 506 were included (vomit during OGTT test was the more frequency exclusion criteria). The prevalence of GD was 11.4%. The ROC curves analysis shows: the best area under curve (AUC) is for 1st VPG measurement (126 mg/dl, AUC 0.885, Se 81.67%, Sp 81.84%, PPV 35.72%, NPV 97.31%), 2nd VPG measurement (102 mg/dl, AUC 0.830, Se 79.31%, Sp 66.07%, PPV 21.54%, NPV 96.54%).
Conclusion: Diagnosing GD through SB would be a usefulness alternative for women with OGTT intolerance: 1st and 2nd VPG present an adequate Se and Sp. We will have a most physiological and better tolerated test to apply in the pregnancy to diagnose the gestational diabetes.
Supported by semFYC

0945

Maternal gestational diabetes and the manifestations of metabolic syndrome in adolescent offspring

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Background and Aims: The increasing epidemiological evidence suggests that the prenatal environment has an important role in the development of chronic adult diseases, like diabetes and cardiovascular disorders. Maternal gestational diabetes (GDM) has been found to associate with subsequent early morbidity of the offspring but there are no population-based studies on the incidence of traits of metabolic syndrome in later life among offspring, and practically no data on gender differences.
Materials and Methods: The general population-based data were obtained from the prospective Northern Finland 1986 Birth Cohort comprising all singleton pregnancies of the 9247 mothers and their offspring born in 1985–86. The latest clinical follow-up was performed at age 16 for the offspring with 74% response rate (N=6798). The risk factor based screening of GDM was performed during pregnancy according to national criteria (glucosuria, prior GDM or macrosomic infant, current macrosomia, BMI>25 kg/m^2, age>40 yr). The diagnosis of GDM was confirmed by 75 g OGTT performed after overnight fasting. The study population with written consent consists of offspring of mothers from three groups: 1) GDM diagnosed by OGTT (n=95), 2) mothers with normal results of OGTT (n=742, screen indicated), and 3) the reference group consisting of mothers without GDM or the above risk factors used for screening (n=3938). Women without OGTT despite indications (n=1942) and women with pre-pregnancy diabetes (n=27) were not included in the analyses. The distributions of metabolic traits were skewed and logarithmic transformations were applied. The results are reported in geometric means and percentage differences between exposure groups with 95% CI.
Results: The prevalence of overweight (BMI>25 kg/m^2) was significantly higher among offspring of GDM-mothers than in the reference group (18.1 vs. 8.4%), and their mean BMI was higher (Table). Waist circumference, insulin and HOMA-S were significantly higher in offspring of GDM-mothers than in the reference group. Interestingly, these findings seem to be stronger in girls than in boys.
Conclusion: The features of metabolic syndrome were clearly seen in offspring of GDM mothers. This risk group should be noted by planning preventive and therapeutic strategies already before potential clinical manifestation of metabolic syndrome.

Outcomes at age 16

	GDM offspring		Reference group				
	n=95		n=3938				
	Mean	95% CI	Mean	95% CI	Percentage difference	95% CI	p-value
BMI kg/m^2	21.9	(21.3, 22.6)	20.6	(20.5, 20.7)	6.4	(3.5, 9.5)	<0.0001
Waist circumference, cm	76.7	(75.1, 78.3)	72.5	(72.3, 72.7)	5.7	(3.5, 8.0)	<0.0001
SBP, mmHg	117	(115, 120)	114	(114, 115)	2.5	(0.3, 4.8)	0.028
DBP, mmHg	68	(66, 69)	67	(67, 67)	1.3	(−0.9, 3.7)	0.251
Insulin, mU/L	10.9	(10.0, 11.9)	9.4	(9.3, 9.6)	15.2	(5.6, 25.6)	0.002
Glucose, mmol/L	5.2	(5.1, 5.3)	5.1	(5.1, 5.2)	1.7	(−0.3, 3.8)	0.101
HOMA-S	475	(435, 519)	548	(540, 555)	−13.3	(−20.6, −5.3)	0.002

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0946

First trimester sex hormone binding globulin and subsequent development of gestational diabetes mellitus

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Background and Aims: Sex hormone binding globulin (SHBG) has been shown to have an inverse association with insulin sensitivity and, thus, its lower value has been suggested as a useful tool for investigating insulin resistance. The association of reduced SHBG with gestational diabetes mellitus (GDM) is now fairly well recognized, but the causal relationship between the two events is yet debated. The present study was undertaken to test the hypothesis that first trimester SHBG may predict the subsequent development of GDM in later stages of pregnancy.
Materials and Methods: A total of 103 subjects, collected from various hospitals and clinics in Dhaka city, were followed from their 1st to 3rd trimesters of pregnancy. Detailed history, clinical examination and anthropometry (height and weight) were recorded. GDM was diagnosed following the WHO guidelines. SHBG level was estimated by Chemiluminescent Immunometric Assay.
Results: Out of the 103, 62 (60%) patients showed normal glucose tolerance upto the end of the pregnancy (the Control

Group). A number of 22 subjects (21%) subsequently developed GDM (the GDM Group) and 19 (18%) patients already showed evidence of glucose intolerance at the first trimester. This last group was designated as Positive Control. Taking 25–75th percentile levels of SHBG in the Control as the range, the reference values for SHBG in our 1st trimester pregnant population was found to be 80–157 nmol/l. The GDM group showed less than 50% of SHBG levels compared to control. [SHBG, nmol/l; median (range): Control, 125 (25–180) vs GDM 52 (15–180), p<0.0001]. About 82% of GDM cases showed SHBG values lower than the lowest limit of the reference range (80 nmol/l) and there was high probability of developing GDM in subjects having low SHBG in the first trimester (Odds Ratio; 14: 95% CL 4.12–48.21). In contrast to GDM the Positive Control Group showed no significant difference of SHBG levels as compared to Control group. Only 36% of the Positive Control subjects showed low SHBG values (ie <80 nmol/l) as compared to 25% in the Control.

Conclusion: The data indicate that insulin resistance has a causal relation with GDM and the abnormality can be detected at the early stages of pregnancy using SHBG as a marker.

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0947

Safety and efficacy of short-acting insulin aspart and lispro analogues in patients with gestational diabetes mellitus (GDM)

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Background and Aim: Because of increased risk of maternal and neonatal complications, insulin treatment is often necessary in GDM women. Although both Aspart (Asp) and Lispro (Lis) have been used systematic comparison with respect to regular insulin (HI) is still scanty. The aim of this study was to compare the pregnancy outcome in GDM women treated with Asp, Lis, or HI.

Material and Methods: In 96 women (age 34.8±4.7 years, 70 primiparous, pre-pregnancy BMI 25.8±5.4 Kg/m2) with GDM (Carpenter and Coustan's criteria at 27.5±2.1 week of gestation) and on 30 Kcal/Kg/die (CHO 50%, proteins 20%, fat 30%) insulin therapy was begun (median value: 29th week of gestation) for fasting/pre-prandial and/or 1-h postprandial glucose>90 and>130 mg/dl (therapy targets), respectively. GDM women were randomly allocated to pre-prandial injection of HI (n=32), Asp (n=31), or Lis (n=33) treatment. Blood glucose profiles (5 points/day) were recorded and weekly contact with the diabetologist ensured for treat-to-target modification of insulin dose, including addition of bed-time NPH insulin if necessary.

Results: All 3 groups were comparable for age, pre-pregnancy BMI, weight gain, and HbA1c. NPH insulin was required in 23 HI (5.6±4 IU), 18 Lis (5.2±5 IU) and 16 Asp (4.7±6 IU) (p=NS). At the end of the pregnancy, total insulin dose (HI 18.4±11, Lis 22.4±13, Asp 22.3±18 UI/day), weight gain (HI 10.6±5.2, Lis 11.2±5.9, Asp 10±4 Kg), fasting plasma glucose (HI 89.6±11.3, Lis 86.1±10.6, Asp 82.6±8.9 mg/dl), and HbA1c (HI 5.45±0.4, Lis 5.35±0.4, Asp 5.45±0.3) were not different. On the contrary, 1-h post-prandial glucose as detrmined after standardized breakfast was higher in HI (135±24 mg/dl) than Lis (119±19 mg/dl, p<0.03) and Asp (121.5±20.3; p=0.06). No hypoglycaemic episode was recorded for any of the 3 treatments. Delivery occurred at 38th week of gestation in all groups. Birth weight was greater in HI (3359±393 vs. Lis 3218±458 and Asp 3066±494 g; ANOVA p<0.04); incidence of macrosomia (LGA) was 15.6% in HI, 12.1% in Lis, and 9.6% in Asp (p=??); in newborns of HI-treated women cranial/thoracic (cc/ct) ratio was lower than Lis and Asp (1.01±0.39 vs. Lis 1.04±0.3; Asp 1.03±0.35; ANOVA p<0.03) with 18% of them with cc/ct <1 (Lis 0%, Asp 3.2%; p<0.02).

Conclusions: In spite of similar overall glycaemic control, use of short-acting insulin analogues in GDM women was ensured better prandial glucose tolerance. This effect was associated with more proportionated foetal growth as compared to use of regular insulin.

0948

Maternal hypoglycaemia and glycaemic control in pregnancy: a randomised trial comparing insulin aspart with human insulin in 322 subjects with type 1 diabetes

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Background: Although outcomes during pregnancy in type 1 diabetes have improved due to intensive insulin therapy and achieving near normoglycaemia, treatment is often complicated by the associated risk of severe and occasion-

ally life-threatening maternal hypoglycaemia. We measured the effect of a rapid-acting insulin analogue in a parallel group, randomised, controlled, multi-centre trial comparing Insulin Aspart (IAsp) and Human Insulin (HI) as meal time insulin in a basal-bolus regimen with NPH.

Material and Methods: Pregnant women with type 1 diabetes, aged 19–43 yr, HbA1c <8% at confirmation of pregnancy, were treated with IAsp (n=157) or HI (n=165). Evaluations included measurements of severe hypoglycaemia (requiring assistance), HbA1c, 8-point plasma glucose (PG) profiles and perinatal outcome.

Results: The estimated risk of severe night-time hypoglycaemia was 52% lower for IAsp than HI (Relative Risk (RR): 0.48, 95% CI [0.20; 1.14]; p=0.10 (gamma frailty model)). Observed rates of severe hypoglycaemia (episodes per year) tended to be lower for IAsp (1.4) than for HI (2.1) and the estimated risk was 28% lower for IAsp than HI (RR: 0.72; 95% CI [0.36; 1.46]; p=0.36). Prandial glucose increments (mean of breakfast, lunch and dinner) were significantly lower with IAsp than with HI both in the 1st (0.73 vs. 1.49 mM; p<0.01) and 3rd trimester (1.10 vs. 1.50 mM; p=0.04). No differences were observed for the average 24-hour PG or HbA1c (HbA1c (%): IAsp: 6.0 and 6.18, HI: 6.04 and 6.26 in 2nd and 3rd trimester, respectively). Most patients in either treatment group showed no progression in retinopathy. Perinatal outcome was generally good, without large differences between IAsp and HI.

Conclusion: The risk of severe hypoglycaemia tended to be lower in the Insulin Aspart treated than Human insulin treated women, especially during the night.

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0949

Safety and perinatal outcome in pregnancy: a randomised trial comparing insulin aspart with human insulin in 322 subjects with type 1 diabetes

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Background: Pregnancy in diabetes is associated with increased complications for mother and offspring, and strict blood glucose control is vital. This parallel group, randomised, controlled, multi-centre trial studied the pregnancy outcome (maternal and foetal) after treatment of pregnant women with Insulin Aspart (IAsp) or Human Insulin (HI).

Materials and methods: Pregnant women with type 1 diabetes, aged 19–43 yr, $HbA_{1c} \leq 8\%$ at confirmation of pregnancy, were treated with IAsp (n=157) or HI (n=165) as meal time insulin in a basal-bolus regimen with NPH. Evaluations included pregnancy outcome, obstetric complications, severe maternal hypoglycaemia and glycaemic control.

Results: Overall, 268 live births and 35 foetal losses (mostly spontaneous miscarriages) were reported (IAsp: 14, HI: 21). No difference in foetal mortality or major congenital malformations was observed between the two groups. The percentage of children who were macrosomic (>4000 g at birth) or large for gestational age was similar in the two groups. Median gestational age was 37.9 and 37.7 weeks, respectively, for IAsp and HI. The most frequent obstetric complications were emergency caesarean section (IAsp: 15.9%, HI: 12.7%), pre-eclampsia (IAsp: 8.3%, HI: 6.7%) and imminent premature labour (IAsp: 3.8%, HI: 4.2%). Preterm delivery was reported in 20% and 30% of live births for IAsp and HI, respectively. Treatment-requiring neonatal hypoglycaemia was reported in 98 of the live births (IAsp: 46, HI: 52). Mean birth weight was 3467 g and 3555 g for IAsp and HI, respectively. The estimated risk of severe maternal hypoglycaemia was 28% lower for IAsp than HI with similar HbA_{1c} values. The lower estimated risk of hypoglycaemic episodes for IAsp was more pronounced for nightly (52%lower) than for daytime (15% lower) episodes; however none of these risk differences were statistically significant.

Conclusion: Treatment with Insulin Aspart was at least as safe as Human Insulin regarding perinatal outcome.

Supported by Novo Nordisk

0950

Congenital anomaly rate in offspring of Italian mothers with type 1 diabetes treated with insulin lispro during pregnancy

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Background and Aims: Some studies indicate that insulin Lispro can be used in pregnant diabetic women based on similar fetal outcomes as those observed in pregnant women using regular insulin.This study aims to analyze Italian data of a multinational, multicenter, retrospective study that included mothers with prepregnancy type 1 diabetes mellitus, who were treated with insulin lispro(L) for at least 3 months before conception and during at least the first trimester of pregnancy, with respect to prepregnant type 1 diabetes mellitus women treated with regular insulin (C) followed from the same centers.
Materials and Methods: 12 Italian centers collected data from pregnant women with diabetes, followed from 1998–2001, including: HbA1c at conception during the first and 3rd trimester,frequency of hypoglycemic episodes, spontaneous abortions, mode and timing of delivery, fetal malformations and/or death. Fisher's Exact test and Wilcoxon test were used to compare proportions and continuous data respectively.
Results: 72 L pregnancies and 298 controls (C), matched for age (31.1+4.8 vs 30.6+4.6), were included in the analysis. 50% of L pregnant had prepregnancy counseling vs 38% of C. During pregnancy, a numerically lower percentage of L patients experienced hypoglycemic episodes than C ones (2.8% vs 5.4%, p=ns). Gestational hypertension and pre-eclampsia were similar in both groups. HbA1c was similar at conception in both groups, improved significantly only in L patients ($p<0.005$) in the first trimester and was similar at the end of pregnancy in both groups. Frequency of cesarean section was similar in both groups(68.1L; 65.4%C). The rate of malformations was similar in both groups (4.2%L; 4.5%C).
Conclusion: The rate of major congenital anomalies was 4.3% [95%CI(0.9%,12%)] for offspring of Italian mothers treated with insulin lispro before and during pregnancy.This rate was not statistically different from a comparable group treated with regular insulin(4.5%). A lower percentage of patients treated with insulin lispro reported hypoglycemic episodes, even with improved glycemic control, as shown by the significant reduction of HbA1c.
These data shows that also in our population the rate of congenital malformations in pregnant women treated with insulin lispro is not different from that observed in pregnant women treated with regular insulin.

0951

Glycaemic control during pregnancy in women with long duration type 1 diabetes: lessons learnt using continuous glucose monitoring systems

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Background and Aims: Recent nationwide studies of women with diabetes show that rates of complications including macrosomia, preeclampsia and prematurity remain very high compared to women without diabetes. Tight glycaemic control throughout pregnancy is important to reduce the risk of complications and current guidelines suggest target fasting glucose levels below 5.4 mmol/L and peak postprandial glucose levels below 7.9 mmol/L. The aim of our study is to use continuous glucose monitoring systems (CGMS) to examine blood glucose levels achieved throughout pregnancy by women with type 1 diabetes of long duration, in particular studying day to day variability and hypoglycaemic excursions.
Materials and Methods: Eight women, with type 1 diabetes of greater than 15 years duration, wore blinded CGMS for up to 7 days at monthly intervals throughout pregnancy during a randomised controlled study of CGMS intervention. For each CGMS study, we determined mean glucose level, percentage readings above 7.8 mmol/L, percentage readings below 3.9 mmol/L and the day to day variability, defined as the difference between highest and lowest 24 hour mean glucose during a CGMS study. HbA1c was measured monthly.
Results: Mean duration of diabetes was 25.6 (16–33) years. The women had 6 (5–8) CGMS studies during pregnancy and mean duration of each study was 4 (1–6) complete days. Seven women were managed with basal/bolus regimens using intermediate insulin once or twice daily as basal insulin. One woman used an insulin pump. Comparing the first CGMS study with the last CGMS study, there was a significant fall in mean blood glucose (8.2(1.9) vs 6.3 (1.4) mmol/L, p=0.04). Hyperglycaemic excursions were seen frequently but between first and last CGMS studies there was a significant fall in percentage glucose readings above 7.8 mmol/L (43.6(15.2) vs 25.2(18.0)%, $p<0.05$). In contrast, there was no significant difference in percentage

glucose readings below 3.9 mmol/L between first and last CGMS studies (17.1(10.5) vs 21.4(13.3)%, NS). Great day to day variability was observed during CGMS studies and there was no significant difference in day to day variability between the first and last CGMS studies (3.4(1.0) vs 2.8 (1.1) mmol/L, NS). The highest day to day variability observed was 5.4 mmol/L. There was a significant decrease in mean HbA1c between first and last studies (7.3(1.0) vs 5.7(0.5)%, p=0.002).

Conclusion: These results show hyperglycaemic excursions remain common throughout pregnancy in women with long duration type 1 diabetes. The data shows that day to day variability of blood glucose is high throughout pregnancy and there is no change in day to day glucose variability or percentage hypoglycaemic readings as pregnancy progresses. The results suggest it is difficult to achieve blood glucose targets with current treatments and may explain the continuing high rate of complications during pregnancy in women with type 1 diabetes.

PS 79

Continuous glucose monitoring

0952

Continuous subcutaneous glucose monitoring in healthy subjects during daily life conditions

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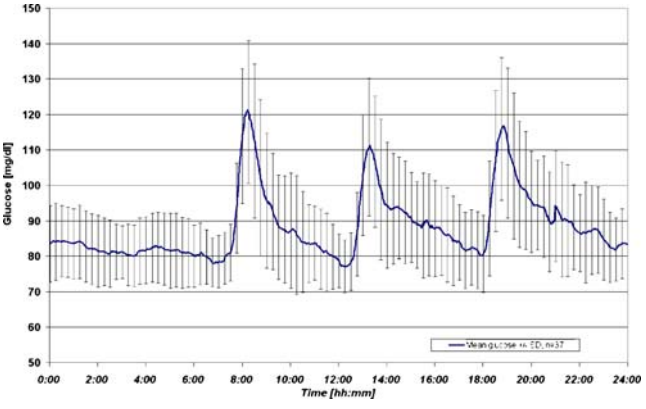
Background and Aims: In recent years, subcutaneous glucose monitoring devices have been introduced into clinical application, and continuous glucose profiles in patients with type 1 diabetes have been investigated in numerous studies. However, therapeutic target ranges for subcutaneous continuous glucose profiles have not yet been defined. Since only little data in healthy persons are available, this study aimed to assess continuous subcutaneous glucose traces in healthy subjects under daily life conditions.

Materials and Methods: In 24 healthy volunteers (12 f, 12 m; age 27.1 (19.4–34.5) years; BMI 22.6±1.7 kg/m2; HbA1c 5.0±0.2% [mean±SD]) an oral glucose tolerance test was performed to exclude diabetes mellitus or IGT. Two continuous monitoring devices based on microdialysis technique (subcutaneous continuous glucose monitoring (SCGM) device: in development by Roche Diagnostics) were applied to the abdominal subcutaneous tissue for ~98 hours; for calibration and verification of tissue glucose values 15 capillary blood glucose measurements were performed per day. During the first two days the subjects stayed in house, while on days 3 and 4 they followed their daily life conditions with free choice meals at fixed times (7:30, 12:30 and 18:00). The continuous monitoring data were calibrated over all reference values. The results of the two glucose monitoring devices were merged to one curve, 37 of 48 twenty-four hour glucose traces recorded during daily life conditions (days 3 and 4) could be evaluated.

Results: The mean 24 h glucose concentration was 88.3±9.5 mg/dl. Mean glucose concentrations in the daytime (6 a.m. to 10 p.m.) and during the night (10 p.m. to 6 a.m.) were 91.0±10.5 mg/dl and 82.8±1.9, respectively (Figure 1). The 3 h and 5 h areas under the curve above baseline were 2908 and 2920 mg/dl*min after breakfast, 3007 and 3931 mg/dl*min after lunch and 3317 and 4275 mg/dl*min after dinner respectively. Time to maximum glucose was 44, 49 and 50 minutes after breakfast, lunch and dinner. Mean preprandial glucose concentrations were 80.8±7.9, 78.3±7.9 and 80.5±10.8 mg/dl, maximum glucose concentrations were 121.2±19.7, 111.3±19.3 and 116.8±20.8 mg/dl for breakfast, lunch and dinner, respectively.

Conclusion: The continuous glucose monitoring data obtained in healthy subjects, provide interesting additional information on fasting and postprandial tissue glucose levels in healthy subjects under daily life conditions. These data may also be used as target continuous glucose traces in the development of closed loop systems using subcutaneous monitoring and algorithm-controlled subcutaneous insulin application.



Supported by Disetronic Medical Systems

0953

Insulin detemir shows a lower glucose variability compared to NPH insulin in subjects with type 1 diabetes undergoing continuous glucose monitoring

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Background and Aims: The aim of the study was to compare the glycaemic profiles of subjects with Type 1 diabetes mellitus treated with either insulin detemir (Detemir) or NPH insulin in a randomised, multi-national, open-label, parallel group trial.
Materials and Methods: Subjects (88 Detemir: 37 NPH) using continuous glucose monitoring system (CGMS), aged≥18 yrs (Detemir: 39.3±10.3 yrs, NPH: 40.9±13.7 yrs), diabetes duration≥1 yrs (17.5±12.1 yrs vs 18.7±10.0 yrs) and HbA_{1c}≤12% (8.3±1.1% vs 8.2±1.3%) were assessed. They were treated once daily with either Detemir or NPH at bedtime for six months, in combination with human soluble insulin (HSI) at main meals. HbA_{1c}, FPG, body weight and within-subject variation were assessed. During the last month of treatment, all subjects wore CGMS for three days and mean amplitude of glycaemic excursions (MAGE) was determined in excess of 1 SD (peak-to-nadir and nadir-to-peak) of the respective 24-hr mean glucose value. Data were presented as mean±SD and frequency (percentage).
Results: After six months, FPG was significantly reduced in the Detemir group (−1.97±6.30 mmol/L, p=0.0059) than in the NPH group (−1.30±6.56 mmol/L, p=0.2364). However, the improvement was comparable between treatment groups (−1.38±0.077 mmol/L, 95% C.I. −2.92;0.152). As for HbA_{1c}, the changes from baseline were not significant for both groups (Detemir: −0.13±0.88%, p=0.1739; NPH: 0.12±1.19%, p=0.5569) and no treatment difference was apparent (−0.2126±0.16%, 95% C.I. −0.526;0.10). Mean daily insulin dose was 0.27 U/kg for Detemir and 0.36 U/kg for NPH. Within-subject variation in self-measured PG was significantly lower for Detemir vs NPH (SD of 2.8 vs 4.1 mmol/L, p<0.001). MAGE was significantly lower with Detemir vs NPH (−1.08± 0.43 mmol/L, 95% C.I. −1.94;−0.227, p=0.0136). The same proportion of patients (76 Detemir: 32 NPH subjects, 86%) reported nocturnal hypoglycaemic events (major, minor, symptoms), 442 vs 219 events.
Conclusion: Subjects with Type 1 diabetes treated for six months with Detemir+HSI experienced comparable glycaemic control, but significantly lower within-subject variability and lower glucose excursions compared to subjects treated with NPH+HSI.
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0954

Diabetes self-management decisions guided by continuous glucose monitoring: Results of an in-clinic pilot study

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Background and Aims: Continuous glucose monitoring (CGM) devices currently available in the United States are approved for use as adjuncts to standard self-monitoring of blood glucose (SMBG). Use of CGM with the STS System (DexCom, Inc.) as a primary means for directing diabetes self-management decisions (SMD) was evaluated in this pilot study.
Materials and Methods: Fifteen (15) subjects with type 1 diabetes participated in staff supervised 10-hour (7 am–5 pm) in-clinic sessions on 3 consecutive days. During all 3 sessions, self-monitoring of blood glucose (SMBG) was performed just prior to each SMD. **Session 1**: CGM blinded, decisions were guided by SMBG. **Sessions 2 and 3**: CGM unblinded (continuous glucose values, trends, and alerts/alarms provided) and was the **only** data used by subjects to guide decisions (SMBG blinded). Every SMD made by the subjects was verified with staff who had access to SMBG data.
Results: Two types of SMD were analyzed: bolus of fast-acting insulin (n=154), and correction of hypoglycemia with carbohydrates (n=43). Of the former, 39 (25.3%) were adjusted by staff prior to delivery, the remaining 115 (74.7%) were unchanged. SMD guided by SMBG (Session 1) vs. CGM (Sessions 2+3) were compared. Each decision was treated independently and deemed appropriate if 3-hours post-decision, the sensor glucose value was within a target range of 80–200 mg/dL (per protocol CGM low/high glucose alert settings). Of SMBG-guided decisions, 45.7% were appropriate; this percentage increased to 64.6% when SMD was guided by CGM (p=0.02). Subjects experienced a median increase of 2.0 hours per session (10 hours) spent in target range during Sessions 2+3, as compared to Session 1 (p=0.003); and reduced time spent>200 mg/dL without increasing time spent <55 mg/dL. No episodes of severe hypoglycemia were reported during this study.
Conclusion: In this pilot study, real-time availability of CGM resulted in an increased frequency of appropriate diabetes self-management decisions with a significant improvement in target range glycemia.

Median Change in Time Spent in Various Glucose Ranges

Glucose Range (mg/dL)	Session 1 vs. Sessions 2+3 (Hrs/Session)	p-value
<55	−0.01	0.6377
55–79	+0.52	0.1937
80–200	+1.99	0.0026
201–300	−2.18	0.0398
>300	−0.19	0.3828

0955

Evaluation of a non-invasive continuous glucose monitoring device in a home use setting

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Background and Aims: A growing body of work supports the benefit of continuous glucose monitoring (CGM) in improving the treatment and health of diabetic patients. Primarily, invasive subcutaneous devices are used to perform such monitoring. The purpose of this study is to evaluate the feasibility of the fully non-invasive NBM-100 (OrSense Ltd.) device for continuous blood glucose (BG) monitoring in type 1 and 2 diabetes subjects.
Materials and Methods: The NBM-100 uses a finger-based sensor shaped as a ring, located at the finger's root. The configuration of this novel sensor enables the detection of red/near-infrared light transmitted through the finger via Occlusion Spectroscopy. Analysis of the resulting spectrum renders the subjects BG levels, Hb/Hct concentrations, and SpO_2 levels. Specifically, the NBM-100 utilizes an enhanced optical signal resulting from a temporary over-systolic occlusion, produced by a finger-based pneumatic cuff. The resulting changes in the optical absorption and scattering create the sensitivity needed for measuring BG and other analyte concentrations. The study consisted of three phases. (1) A hyperinsulinemic hypoglycemic clamp was performed at a clinic in 4.5 hour sessions (2M, 3F, aged 21–40, DM1) with reference BG measured every 5 minutes (Freestyle, Abbot). (2) A home use trial conducted on 17 subjects (8 DM1, 9 DM2 10M, 7F, ages 21–74) with 2–10 days of 8–16 hour sessions. Reference BG readings were collected every 30–60 minutes (Glucose 201+, HemoCue). (3) A 24 hour use trial with 4 subjects (2M, 2F, aged 21–40, DM1), conducted in a clinic with venous reference BG readings collected every 20 minutes (Olympus AU 2700). In all three trial settings the non-invasive data was collected every 10 minutes. In each session, glucose predicting parameters were calibrated using four BG reference points taken within a three hour period. Based on a predetermined inclusion criterion from the calibration phase, 88 of the 101 sessions were included in the above analysis.
Results: A prospective analysis was performed on the NBM-100 readings for all 88 patient sessions to calculate the standard error values for the total of 1202 paired data points (NBM-100 vs. Reference Gl). The glucose range was 38–488 mg/dl. The resulting median relative absolute error (RAE) was 12.6%, the median absolute error (MAE) was 21.2 mg/dl, and the overall correlation was r=0.87. A Clark error grid analysis showed that 94.8% of the measurements fall within zones A and B.
Conclusion: This study indicates the potential use of the NBM-100 as a non-invasive sensor for continuous BG evaluation. In all three clinical settings user compliance was positive. The NBM-100 is currently under evaluation in broader clinical settings.

0956

Clinical trial of a seven-day continuous glucose sensor demonstrates the importance of fasting hyperglycaemia and the dawn phenomenon

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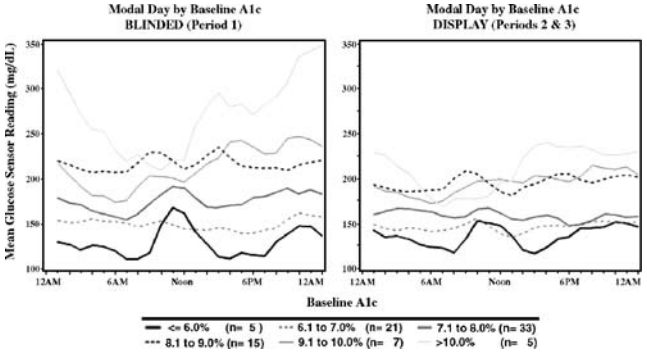
Background and Aims: Relative contributions of fasting and post-prandial hyperglycaemia differ at different levels of glycaemic control (HbA1c values). Previous studies have documented higher contributions of post-prandial elevations at lower HbA1c values based on self home blood glucose monitoring (SMBG). No data is available on continuous home glucose monitoring (CHGM) and its relationship to different HbA1c values. We evaluated this relationship in this study.
Materials and Methods: Sixty-nine subjects with type 1 diabetes and 17 with insulin-requiring type 2 diabetes participated at five centers. Baseline HbA1c was measured within 30 days of enrollment. Subjects inserted and wore a transcutaneous glucose sensor (STS System, DexCom, Inc.) for 7 days over 3 consecutive periods during normal activities. Sensors were BLINDED for period 1. During periods 2 and 3 (DISPLAY), glucose values, trend graphs, and high/low alerts and alarms were provided.
Results: Of 6357 paired sensor-SMBG points prospectively analyzed, 97.2% fell in Clarke Error Grid zones A+B with none in zone E; mean absolute relative difference was 15.7% with stable performance over 7 days. Continuous glucose values were pooled for subjects within 6 groups of baseline HbA1c (from <6% to>10%, increments of 1%). Modal day graphs for each HbA1c group were generated by

plotting mean hourly glucose values during BLINDED and DISPLAY device use (Figure). During BLINDED use, distinctive glycaemic patterns were observed at varying baseline HbA1c levels. Of note, mean glucose values from 4–7AM (dawn phenomenon and fasting) were only normal for subjects with HbA1c <6%, and mean glucose values between 4PM and 12AM were higher by ~20–40 mg/dL per 1% increase in baseline HbA1c ($p<0.05$ when comparing the HbA1c <6% group to each of the 3 highest HbA1c groups at these hourly time points). Glycaemic excursions were improved during the DISPLAY periods.

Conclusion: We conclude that 7-day use of the STS System was safe, accurate, stable, and resulted in improved glycaemic excursions. Continuous glucose monitoring, with a focus on fasting and evening hyperglycaemia and the dawn phenomenon, may help achieve lower HbA1c values.



Supported by DexCom, Inc.

0957

Home use evaluation of the freestyle navigator continuous glucose monitoring system

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Background and Aims: The FreeStyle Navigator Continuous Glucose Monitoring System, an investigational continuous glucose monitoring system, was tested by 137 subjects with type 1 and type 2 diabetes to validate its use in the home environment at 6 clinical sites. Its usability was assessed by continuous use for 40 days, followed by a user questionnaire on ease and potential benefit of use. The system includes a sensor (worn up to 5 days), a reusable transmitter, and a handheld receiver with a built-in FreeStyle® blood glucose (BG) monitoring system. Each sensor requires calibration against capillary BG at approximately 10, 12, 24, and 72 hours after insertion.

Materials and Methods: Sensor insertions at either arm or abdomen (minimum of 8) and calibrations were performed by the subjects. Real-time data and alarms were not available for the first 20 days (blinded phase). Real-time data, trend arrows and threshold alarms for hypo- and hyperglycemia (programmed at 65 mg/dL and 300 mg/dL, respectively) were unblinded for the next 20 days. For the entire study, subjects were instructed to make all treatment decisions based on capillary BG tests only and to perform BG tests with the built-in FreeStyle meter in duplicates prior to meals, at bedtime, and when symptomatic for hypo- or hyperglycemia at a minimum.

Results: Summary data represents 123 subjects (mean±SD, age: 48.6±13.0 years, duration of diabetes: 22.0±11.9 years, daily insulin use: 51.7±28.7 IU) who completed the protocol. Based on 11,487 paired FreeStyle Navigator system/BG data points, the mean absolute relative difference was 14.4±13.4%. A total of 14 subjects withdrew during the first phase of the study due to non-compliance with protocol demands (n=8) or technical difficulties handling the device (n=6). The majority of subjects reported that system functions were easy to learn: 90.5% age 18–34, 84.8% age 35–54, and 93.0% age 55+. Overall, 93.7% of the subjects found the information provided by the FreeStyle Navigator system helped them understand their blood glucose, and 93.6% felt glucose alarms were easy to interpret. When asked about the benefit of continuous glucose data, 100% of subjects age 18–34, 95.4% age 35–54, and 93.0% 55 and over responded that it was simple to understand glucose changes.

Although not considered a primary endpoint, subjects spent significantly more time in euglycemia in the unblinded phase. Subjects with type 1 spent significantly less time (0.8 hours versus 1.4 hours, or 42% less) in the hypoglycemic range, defined as <70 mg/dL, whereas subjects with type 2 shortened significantly the time spent in hyperglycemia (8.0 hours versus 9.6 hours, or 17% less), defined as>300 mg/dL, with no increase in hypoglycemia.

Conclusion: Accurate sensor performance coupled with general ease of use and interpretation of continuous glucose data demonstrate safe and efficient use of the FreeStyle Navigator system in the home environment. The observed improvements in glycemic control reinforce the clinical utility of the FreeStyle Navigator system.

Supported by Abbott Diabetes Care, Abbott Laboratories

0958

Clarke Error Grid and Continuous Clarke Error Grid analysis of the accuracy of the 5-day FreeStyle navigator continuous glucose monitoring system

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The FreeStyle Navigator Continuous Glucose Monitoring System is an investigational continuous glucose monitoring system based on Wired Enzyme™ glucose transduction technology. The FreeStyle Navigator system includes a disposable subcutaneous sensor that can be worn up to 5 days, a radio transmitter connected to the sensor, and a small handheld receiver.

The accuracy and performance of the sensor were evaluated in a multi-center, prospective, single-arm study. Fifty-eight subjects aged 18 to 64 were enrolled, and each wore two sensors, one on the arm and one on the abdomen. Sensors were calibrated with capillary BG measurements using the built-in FreeStyle® blood glucose (BG) monitoring system at approximately 10, 12, 24, and 72 hours after insertion. The FreeStyle Navigator system measurements were collected at one-minute intervals for the entire study and were not displayed to patients or investigators. Venous blood glucose measurements were taken using a standard laboratory reference (YSI 2300) every 15 minutes for 50 hours distributed across the 5-day sensor wear.

The primary endpoint was accuracy as determined by pairwise comparisons of the FreeStyle Navigator system values and laboratory reference values. These measurements were compared using mean and median absolute relative difference, standard Clarke Error Grid Analysis, and Continuous Glucose Error Grid Analysis (CG-EGA), a method of evaluation that assesses both accuracy for point values and for measurements of direction and rate of change.

Regarding precision, the matched sensors worn on the arm and abdomen had a coefficient of variation of 10% (n=312,953). The analysis yielded a mean absolute relative difference of 12.8% and a median absolute relative difference of 9.3% between the FreeStyle Navigator system values and YSI.

On the Clarke Error Grid (CEG) 81.7% were in zone A and 16.7% were in zone B. The data included periods of high rates of change of blood glucose during IV glucose and insulin challenges with periods of hyperglycemia and hypoglycemia. When the rate of change of blood glucose was between −1 mg/dL/min and+1 mg/dL/min, the percentage in zone A increased to 88%. The accuracy performance remained high through all five days of use, with 82.5% of data in zone A on the first day and 80.9% in zone A on the fifth day.

Continuous Glucose Error Grid Analysis (CG-EGA) yielded 81.1% in the rate error grid zone A, 14.4% in the rate error grid zone B and 1.5%, 2.3% and 0.7% in the rate error grid zones C, D and E respectively. Point analysis using the CG-EGA yielded 85.7% in point error grid zone A, 13.6% in point error grid zone B, 0.0%, 0.7% and 0.0% in point error grid zones C, D and E respectively. The CG-EGA analysis combining rate and point information revealed that accuracy, measured as a percentage of accurate readings plus benign errors, was 97.5% (94.1% accurate, 3.4% benign).

The study results support the accuracy and clinical performance of the FreeStyle Navigator continuous glucose monitoring system over five days of sensor wear.

Supported by Abbott Diabetes Care

0959

An innovative approach to non invasive glucose monitoring

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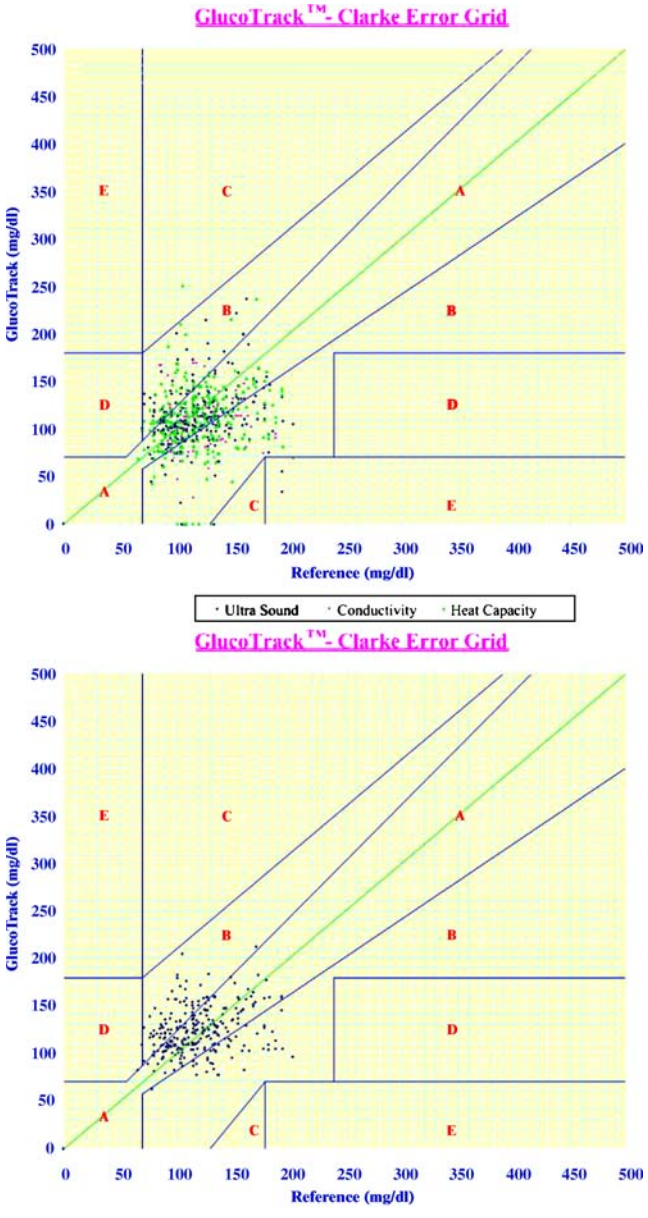
Background and Aims: Attempts to develop an accurate non-invasive glucose monitoring device have been only partially successful. Depending on the particular method, the readings might vary with changes in glucose level, but might also be affected by changes in other components in the blood. In order to achieve an accurate reading, there is a need for a mechanism which will idle the impact of the non-glucose components and isolate the changes caused by the glucose per se.

Materials and Methods: An innovative approach to the problem is the simultaneous utilization of three different technologies and comparison of the separate results by using a unique algorithm. For each glucose reading, achieved by one method, a tolerance window of±N% is opened around the original value. The three tolerance windows are then correlated and compared, while overlapping is expected. If there is a full correlation, meaning three overlaps are achieved, then the final result will be a weighted average of the three measured values. If only two overlaps occur, then the tolerance windows are shrunken, (0.75 * N) and an overlapping between the two readings is then checked. Failure of overlapping causes a reading error and auto repeat of the measurement. Three trials are allowed. Failure in all three attempts brings an error message, requesting a new personal calibration.

Results: ***GlucoTrack***™ uses Ultra Sound, Conductivity and Heat Capacity technologies for measuring the glucose level.

A basic tolerance window of N=20% was used in 278 measurements. As a reference we used the FreeStyle® invasive glucose measurement device. The results are shown below: raw glucose readings of the three different technologies are shown in the left Clarke Error Grid (CEG), while the final results, after using the algorithm are demonstrated in the right CEG. The enhancement in precision is clearly shown in the right CEG. The device has been used by 69 patients. A total of 174 points were measured: 83.3% fell in Zone A of CEG and 16.7% in Zone B.

Conclusion: Using a combination of three different technologies and comparing the results by the suggested algorithm improves the overall accuracy level of the glucose measurement, thus ***GlucoTrack*** is a novel non-invasive device for the accurate measurement of blood glucose.



0960

Fiberoptic hybrid sensor for continuous glucose monitoring

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Background and Aims: Continuous glucose monitoring (CGM) is beneficial for tight glycaemic control in diabetic as well as in critically ill patients. Most of the sensors used for CGM are based on the glucose oxidase reaction and electrochemical detection of H_2O_2. Such sensors often suffer from poor selectivity and effects caused by changing pO_2-levels. This is of particular importance in the case the measurement is performed in critically ill patients (high doses of drugs) and in the interstitial fluid (low and varying pO_2-levels).The objective was therefore to design and test a new sensor approach using optical hybrid sensors for simultaneous measurement of oxygen and glucose minimizing the effects of interfering substances and changing pO_2-levels.

Materials and Methods: The hybrid sensor consists of two oxygen optodes (each 125 µm in diameter) whereof one optode acts as glucose sensitive probe and the other one as reference - measuring the surrounding oxygen tension. Both measurements are based on the phase shift method for the determination of the luminescence decay time, which is insensitive to changes of the absorption of the luminescence. Long-term stability, accuracy and precision were determined, sensor response to interfering substances and different physiological pO2 levels were investigated in-vitro.

In-vivo a flow through configuration of the sensor was combined with the microdialysis technique. This approach was tested in 16 (12 healthy, 4 diabetic) subjects over a period of 24 hours. Meals were standardized; blood readings were taken in 15 minutes to hourly intervals and compared with the sensor signals.

Results: During in-vivo trials sensor drift was negligible; in Clark-Error Grid analysis more than 96% of the measured values were in region A or B. 20% of the sensor data had to be discarded for technical reasons (mainly fluidic problems).

In-vitro sensor accuracy was between 2% and 15%, precision <2% and drift within 72 hours <5%.

Sensor response to 6 most common interferences:

Interfering Compound	Therapeutic/ Physiological Level [mg/dl]	Test Concentration [mg/dl]	Interference Effect at 5 mM glucose [mM]
Ascorbic Acid	0.8–1.2	3	<0.1
Acetaminophen	1–2	20	<0.1
Acetylsalycylat	15–30	50	<0.1
Uric Acid	7	20	<0.1
Mannit	not available	200	<0.2
Dopamine	0.4–1.6	13	<0.1

Conclusion: The new sensor approach enables simultaneous measurement of oxygen and glucose which results in stable glucose measurements. In addition the optical measurement is very insensitive to interferences which turns out to be beneficial in critically ill patients.

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PS 80

Inpatient glycaemia/diabetes management

0961

A comparison of capillary and venous blood glucose concentrations measured during steady and dynamic glycaemic states

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Background and Aims: A February 2006 AACE/ADA consensus conference on inpatient diabetes and glycemic control identified glucose sampling sites and rates as an area needing further research. We compared capillary and venous blood glucose concentrations during euglycemic, hypoglycemic, and hyperglycemic steady states and transition periods (dynamic states) between the steady states.

Materials and Methods: Forty (40) patients with Diabetes Mellitus (28 with Type 1 and 12 with Type 2 Diabetes, 25 male, 15 female, age 38±12, BMI 27±4.1) participated in this glucose clamp study. Experimental intervention began with the subject in a euglycemic steady state (~120 mg/dL). A Biostator was used as glucose clamp device over a 3–8 hour period to achieve hypoglycemic (~50 mg/dL), hyperglycemic (~250 mg/dL), and hypoglycemic (~50 mg/dL) plateaus in each subject and to induce the dynamic states between the plateaus. Plateaus were kept for approximately 30 minutes, the rate of change during the dynamic states was at least 2 mg/dl/min. Capillary (fingertip) and venous (forearm) samples were drawn at the beginning of the experimental intervention, at the beginning and end of each plateau, and at the approximate midpoint of each transition period (total of 10 sampling points). Plasma glucose concentrations of the capillary and venous samples were determined with the YSI 2300 Blood Glucose Analyzer. Results were analyzed in two ways: a) for each of the sampling points, the mean capillary and venous glucose concentrations were calculated, and b) for each subject and sampling point, the difference between capillary and venous concentrations was plotted against the venous concentration (Bland-Altman plot).

Results: Mean glucose concentrations followed the pattern of 1) decrease, 2) steady state (hypo), 3) increase, 4) steady state (hyper), 5) decrease, and 6) steady state (hypo). Mean capillary and venous concentrations were nearly identical during phases 1, 2, and 6; during phases 3 and 4, capillary glucose exceeded venous glucose by approximately 6–14%, and during phase 5, venous glucose exceeded capillary glucose by 5%. Bland-Altman analysis (see table below) shows a similar relationship: during the periods of decreasing blood glucose, venous concentrations tended to be higher, and during the period of increasing blood glucose, venous concentrations tended to be lower.

Conclusion: Our data suggests that capillary or venous blood may be used for sampling under steady state conditions. Samples collected when glucose levels are rapidly changing frequently show large site-to-site differences. Protocols to guide inpatient mangement and assess glucose meter performance should account for these potential differences.

Diff [Cap-Ven] (mg/dL)	Steady State (n=270)		Dynamic State [decr.] (n=75)		Dynamic State [incr] (n=39)	
	n	%	n	%	n	%
>+15	31	*11.5*	8	*10.7*	23	*59.0*
+10 to+15	18	*6.7*	1	*1.3*	3	*7.7*
+5 to+10	40	*14.8*	8	*10.7*	1	*2.6*
−5 to+5	155	*57.4*	26	*34.7*	6	*15.4*
−5 to−10	15	*5.6*	9	*12.0*	2	*5.1*
−10 to−15	8	*3.0*	6	*8.0*	1	*2.6*
<−15	3	*1.1*	17	*22.7*	3	*7.7*

Supported by LifeScan, Inc.

0962

Assessment and validation of a novel critical pathway for the management of hyperglycaemia in cardiac patients admitted to the cardiac care units

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Background and Aims: Morbidity and mortality in hospitalized patients is increased in hyperglycemia. Normalization of blood glucose levels using intensive insulin infusion protocols has been shown to improve clinical outcomes. Although Insulin infusion algorithms have recently been published, a major obstacle in their implementation is their complexity. We have developed a straightforward, novel pathway for the management of hyperglycemia which overcomes these technical difficulties.
Materials and Methods: This pathway incorporates two new concepts - the "WHEEL" concept for insulin dosing, complemented by the notion of "Catch-up Insulin". The WHEEL which serves as a treatment guideline is made up of four circles depicting the range of blood glucose on the innermost circle and three tiers of intravenous insulin infusion rates on the outer circles. The graphic WHEEL is then translated into a nursing work sheet. Cardiac Care Unit (CCU) nurses are able to follow the protocol without any further assistance. We tested the protocol on 10 patients who were admitted to the CCU at St. Luke's-Roosevelt Hospital of Columbia University in New York, aiming for tight blood glucose (BG) control with a BG range between 80 and 120 mg/dl measured by finger sticks (FS). Secondarily we aimed to show that the implementation of the WHEEL concept would allow us to achieve target range BG control without the risk of hypoglycemia. The control group consisted of 10 patients managed with "standard" insulin drips adjusted based on physician determination prior to the pathway implementation.
Results: 10 patients in the Control group (CG) (60.8± 16.9 years, 40% males) and 10 patients in the On-Protocol group (PG) (63.6±12.5 years, 60% males), $p=0.67$, were compared. The mean initial glucose was (369±162 mg/dl) & (329±157 mg/dl) in the CG and PG respectively. The mean blood glucose on treatment in the CG was (157±53 mg/dl) compared to (117±17.5 mg/dl) in the PG, $p=0.06$. Target blood glucose levels were reached in 30% of the CG and 70% of the PG $p<0.001$. The lowest BG readings in the CG were (46.2±21 mg/dl) vs. (82±10.4 mg/dl) in PG, $p=0.0003$. The incidence of symptomatic hypoglycemia was (0%) in the PG compared to (90%) in the CG, $p<0.0001$.
Conclusion: This pilot study shows that our concept and implementation of the WHEEL is an easily applicable IV insulin protocol that can be managed by a CCU nurse and will facilitate the accomplishment of intensive glycemic control in all cardiac critical care patients without causing a significant number of hypoglycemic episodes.

0963

Algorithm changes in computerized IV insulin infusion protocol using the clarian glucostabilizer (CGS©) result in marked reduction in incidence of hypoglycaemia

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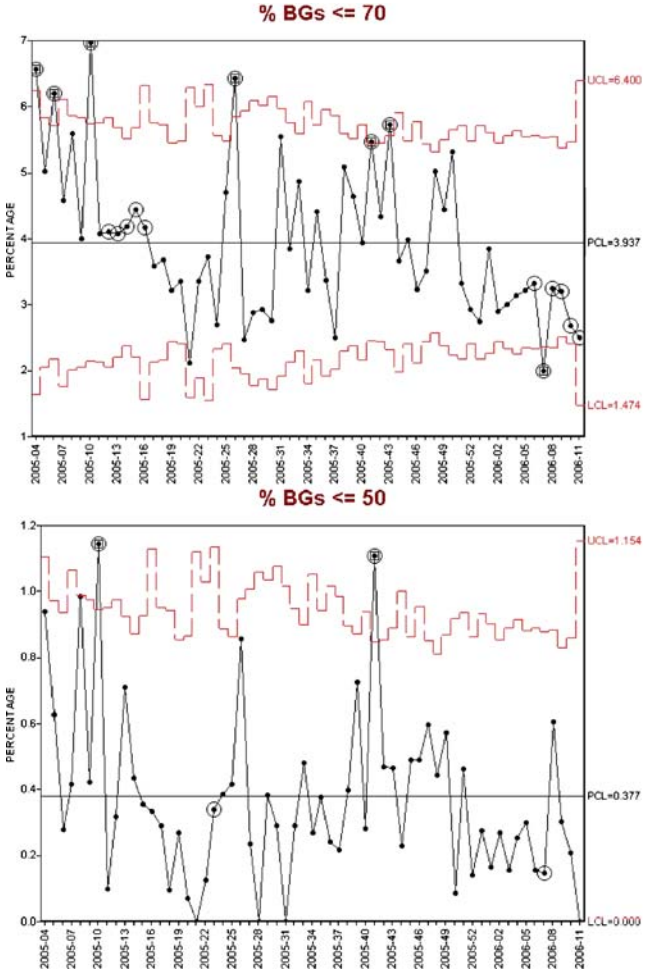
Background and Aims: We have previously shown the effectiveness of a computerized IV Insulin (IVI) protocol in achieving and maintaining Blood Glucose (BG) targets. Once the physician orders IV insulin with a target BG range, the IVI program recommends an insulin infusion rate followed by an alarm for BG checks every 60–120 minutes. Once a new BG is entered, the program recommends change/ no change in the insulin infusion until target BG is reached.
Materials and Methods: This study focused on cardiovascular surgery patients, whose target BGs generally are 80–110 mg/dl in our institution. With this protocol, average hypoglycemia rate (BG≤70 mg/dl) was 4.25%, with 0.42% of BGs being <=50 mg/dl. To attempt to further decrease hypoglycemia while on the protocol, we instituted the following changes in the algorithms used in the Clarian Glucostabilizer (CGS©) Protocol: If a patient had 3 out of the last 8 blood glucose values≤70 mg/dl, the infusion rate was cut in half and the target blood glucose range was raised by 10 mg/dl at the lower and upper limits (e.g. target of 80–110 mg/dl was changed to 90–120 mg/dl). These changes were instituted in late Dec 2005 (data point 2005–52).
Results: For % BG≤70 mg/dl, with the above process modification, we were able to show a statistically significant 30% reduction in the hypoglycemia rate from 4.25% to 2.99% (see "p" Control Chart; %BGs <=70). For % BG≤50 mg/dl, the hypoglycemia rate was reduced by 43%, from 0.42% to 0.24%. There were some readings in week 2006–8, when there were a total of 8 events with BG≤50 mg/dl, which skewed the statistical significance for this BG level, but overall the trend appears to be towards marked reduction in the hypoglycemia frequency (see "p" chart, %BGs <=50).
Conclusion: Programming an algorithm change of a reduction in the infusion rate and slightly less aggressive BG targets in patients on IV insulin, results in a marked decrease in the frequency of hypoglycemia thus increasing

the safety of the Clarian Glucostabilizer©, a computerized IV insulin Protocol.



0964

Use of Glucommander following cardiovascular surgery resulted in ideal control in 96% of patients and 1.1 day reduction in LOS

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Background and Aims: Because the IHI has set a standard of keeping all perioperative BGs <200 mg/dl following CV surgery and because our CVsurgery unit was only achieving this goal in 56% of patients during the past year, we have initiated a new protocol for BG management.
Materials and Methods: The protocol was developed by a committee of surgeons, nurses, and endocrinologists. The basis of this protocol is the Glucommander. This is a computer program advising IV insulin dosing which we have developed. Glucommander has been extensively studied in various clinical situations. The goal of the protocol is a mean BG of 105 mg/dl with minimal hypoglycemia and post-op BG's <200 mg/dl.
Results: At this point 182 CV surgery patients in Piedmont Hospital have been studied. The mean A1c of all patients was 6.4%. 31% were recognized as having diabetes on admission. The criteria for treating patients with IV insulin was a history of diabetes, BG>140 mg/dl, or two BG's post-op>110 mg/dl. 91% of patients were placed on IV insulin. They stabilized at a BG <120 mg/dl in an average of 3.2 hrs. IV insulin was continued for an average of 29 hrs. 96% were controlled so that no BG was>200 mg/dl in the 48 hours following surgery. Only 8% had transient BG <60 mg/dl (51–58 mg/dl). 13% of patients were on insulin on admission. 53% were transitioned to sc insulin while in the hospital. 34% were discharged on insulin. All were on basal-bolus insulin with glargine and aspart following IV insulin and following discharge.
The LOS post-op in this population was 6.2 days. This compares to the LOS in the year prior to initiation of this protocol of 7.3 days and projects to a saving of $2.1 millions per year in one 450 bed hospital.
Conclusions: This study has shown that it is possible to safely achieve and maintain normoglycemia following CV surgery in patients who have been on cardiopulmonary bypass and treated with 250–500 mg methylprednisolone and ionotropes. This excellent BG control is expected to reduce post-op infections and complications to levels comparable to non-diabetic persons and not only reduce LOS but decrease readmissions.

0965

Effect of nutritional protocol and measurement error on automated glucose control in the ICU

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Background and Aims: Tight glycaemic control has been shown to reduce mortality and morbidity in critically ill patients. Using *in silico* computational approach, the objective of this study was to evaluate the effect of nutrition and the measurement error (ME) on the quality of glucose control with fully automated computer-based algorithm.
Materials and Methods: *In silico* simulation environment was used to simulate a 48 h clinical trial with a computer-based control algorithm in the intensive care unit (ICU). The simulation environment represented all components of a clinical trial such as the experimental protocol, synthetic subjects (a glucoregulatory model with patient-specific parameters), a glucose meter with a defined ME, an insulin pump, a glucose controller, and finally, statistical measures to describe

the results. A virtual ICU population of 21 subjects was used to simulate clinical trials using two types of nutritional protocols, simple and complex, and various levels of the ME. The simple protocol consisted of a constant parenteral glucose infusion of 4 g/h and a 40 g CHO meal at 44 h. The complex protocol consisted of varied parenteral and enteral glucose infusions, a parenteral glucose bolus of 12 g at 15 h and a meal of 40 g CHO at 44 h. The parenteral glucose infusion commenced at a rate of 4 g/h at the beginning of the study. The rate was increased to 8 g/h at 12 h and dropped back to 4 g/h at 24 h. At 30 h the infusion rate was increased to12 g/h and continued until the end of the study. The enteral glucose infusion commenced at 33 h and 20 min at a rate of 8 g/h and continued until the end of the study. Glucose was sampled hourly. The coefficient of variation (CV) of the ME ranged from 2.5 to 15%. A number of clinical and statistical measures were used to assess the quality of glucose control.

Results: The mean glucose, time spent in target glucose band, the hyperglycaemic index and the number of hypoglycaemia episodes versus the two types of nutritional protocol and the selected levels of measurement error are listed in Table 1. The measures deteriorated when moving from the simple to the complex protocol. A significant deterioration was also noted with the increasing level of the ME. Severe hypoglycaemia episodes (<2.8 mM) were observed with the ME>10%.

Conclusion: With automated glucose control, nutritional protocol should be kept simple to facilitate an effective control of glucose. The measurement error of the glucose measuring device should not be higher than 10%.

Table 1 Control measures for simple and complex protocols and selected levels of ME (N=21)

	Simple Protocol				Complex Protocol			
	2.5%*	5%	10%	15%	2.5%	5%	10%	15%
Mean Glucose [mM]	6.00	5.92	5.97	5.94	6.08	6.12	6.25	6.53
Time in Band [%]**	80	69	61	51	53	51	38	29
HGI [mM]#	0.23	0.24	0.33	0.49	0.50	0.48	0.60	0.89
Hypo Episodes (<3.3 mM) [unitless]	0	1	2	11	1	0	0	3
Severe Hypo Episodes (<2.8 mM) [unitless]	0	0	0	2	0	0	0	0

*ME expressed as CV
**Target glucose band 4.4 to 6.1mM
Hyperglycaemic index

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0966

Structured approaches to the evaluation and treatment of inpatient hyperglycaemia and length of stay in the hospital

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Background and Aims: The ***S***ystematic ***U***tilization of ***G***lucose ***A***ssessment and ***R***esponse (SUGAR) Program was implemented in a staged manner to reduce inpatient hyperglycemia at Clarian Health Partner Hospitals in Indianapolis, USA.

Materials and Methods: The program electronically tracks all in-hospital Blood Glucose (BG) measurements and triggers intervention (if BG is>180 mg/dl;>110 mg/dl in the ICU). In the ICU, target BG is achieved with IV insulin (IVI) driven by a computer program prompting BG monitoring and IVI adjustment to target. In non-ICU settings, BG control is driven by ongoing programs of increasing awareness of inpatient hyperglycemia through education (morning reports/computer training modules) for physicians/nursing staff by inpatient diabetes nurses and standardization of s/c insulin orders.

Results: We have previously reported significant increase in the numbers of patients reaching target BG from 75% BG<180 mg/dl to 83% (p<0.001) with a simultaneous increase in the number of BG tests from~ 40,000 to over 65,000/month. The main impact has been with ICU targets being achieved through our IVI program (started Oct 2004) with over 60% now achieving target BG (up from 26%). In non-ICU patients we have not seen substantive reduction in overall BG targets, although there has been a sharp increase in endocrinology consults for diabetes. This study analyzes the length of stay (LOS), charges and mortality data from the hospital database during this time (data from patients≥18 years of age and with>one BG measurement during hospitalization).

See Table 1 data for ALL patients (p<0.001:Jan-Jun 2005 vs Jul-Dec 2005 for LOS and charges; mortality p=0.013.Other time points mortality p<0.001compared to Jul-Dec 2005).

See Table 2 data for patients with at least one day in ICU (p<0.001:Jan-Jun 2005 vs Jul-Dec 2005 for LOS and charges; p=ns for mortality)

Conclusion: Our analysis shows that LOS decreased during the period when tight glycemic control (TGC) measures were introduced in the hospital accompanied by a reduction in charges despite a sharp increase in the number of glucose measurements. However, since overall BG control has improved only in ICU's, further studies are

required to evaluate the direct effects of glucose reduction versus other factors that might be contributing to this reduction in LOS.

Table 1: LOS All Patients

Months	# of Patients	Mean LOS (Days)	Charges ($)	Mortality (%)
Jan-Jun 04	13078	8.7	34661	4.5
Jul-Dec 04	13706	8.5	34900	4.4
Jan-Jun 05	13882	8.4	37836	4.2
Jul-Dec 05	13139	7.7	35538	3.6
TOTAL	**53805**	**8.3**	**35734**	**4.2**

Table 2: LOS ICU Patients

Months	# of Patients	Mean LOS (Days)	Charges ($)	Mortality (%)
Jan-Jun 04	3209	13.2	70652	11.0
Jul-Dec 04	3220	13.5	72762	11.8
Jan-Jun 05	3105	13.2	80478	11.6
Jul-Dec 05	2865	11.5	72421	10.4
TOTAL	**12399**	**12.9**	**74078**	**11.2**

Supported by Novo Nordisk Pharmaceuticals

0967

Effectiveness of acute diabetes complications management in day hospital care in new onset type 1 diabetes mellitus patients

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Background and Aims: Acute metabolic complications in new onset type 1 diabetes have been treated in a conventional hospitalization ward. The inhospital management is an expensive way of care, but there are no studies comparing effectiveness and feasibility of Day Hospital treatment versus conventional hospitalization for adults with type 1 diabetes. Aims: 1.To assess the effectiveness of treating acute complications of diabetes (ACD) in a day hospital (DH) versus conventional hospitalization (CH). 2. To evaluate long term (1 year) glycemic control in both groups. 3. To compare short term re-admissions (3–6 months) due to ACD between study groups.

Material and Methods: Study design: prospective, non randomized, cohorts study with 1 year follow-up.
We included all adults with new onset type 1 diabetes (positive GAD Ab, C-peptide <1.0 ng/mL) consecutively admitted in our hospital, with ACD (ketosis/ketoacidosis or acute hyperglycemia>300 mg./dl).
We excluded patients presenting HCO3 <10 mEq, glycemia>600 mg/dl, severe intercurrent illness (infectious, CV disease....) and social isolation.
Patients were assigned to DH or CH according to time of admission to the emergency-room. If patients were admitted out of DH opening hours (Monday through Friday 08:00–17:00 h) they were assigned to CH. All patients admitted in CH cohort could have been assisted in DH if they had arrived during the DH-opening hours. Investigators did not participate in group assignment.
The cost calculation included structural cost, and all visits (emergency ward, Day hospital, ambulatory consultations).
Results: During the period studied (2000–2004), fifty patients met the inclusion criteria, and in all cases the ACD was the first manifestation of new onset type 1 diabetes. The average age was 29,98 years (range 15–57 years), ketoacidosis/ketosis was present in 74% of the patients (37 cases) and 26% (13 cases) had acute hyperglycemia.

	Day hospital	Hospitalization	
n	32	18	
Age (years)	31,8+10,6	26,8+9,2	n.s
Gender (M/F)	19/13	12/6	n.s
Blood Glucose (mg/dL)	362,5+111,8	379,39+99,5	n.s
PH	7,34+0,07	7,34+0,06	n.s
HCO3 (mmol/L)	24,1+7,4	24,4+4,8	n.s
Length of stay (days)	0	6,83+1,30	<0,001
HbA1C (baseline)	11,61+2,40	11,72+2,2	n.s
HbA1C (3 months)	6,56+0,85	6,95+0,88	n.s
HbA1C (6 months)	6,61+1,17	6,48+0,77	n.s
HbA1C (1 year)	6,93+1,43	6,70+1,21	n.s
Hypoglycemic episodes at 3 months	4,79+2,28	4,75+4,35	n.s

There were no differences in the number of short term re-admissions (at 3–6 months) when comparing DH and CH cohorts (1 patient in each group). After discharge the mean number of ambulatory visits per patient over a six months period was similar in both groups (7,5±1,5 in DH and 6,8±4,1 in CH). The median cost per patient and year was 584,9 € for the DH cohort and 1.796,0 € for the CH group.
Conclusions: Our study seems to confirm DH management shows a clear advantage. Results show a net economic saving per assistance of 1.211,1 € with no differences in short and long term glycemic control nor in the number of re-admissions.
Supported by FIS 020402 (Spain Department of Health)

PS 81

Continuous insulin infusion - subcutaneous or intraperitoneal

0968

Alternate site (like) phenomenon: rapid changes from hypoglycaemic to hyperglycaemic glucose levels in a clinical-experimental study in patients with type 1 diabetes

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Background and Aims: Monitoring of glucose changes by SMBG and by continuous glucose monitoring (CGM) systems is prone to certain delays during rapid changes in glycemia if the blood sample is collected at non-glabrous skin or in deeper layers of the skin. Aim of this study was to evaluate the magnitude of such delays.
Materials and Methods: We performed a clinical experimental study with a glucose-clamp day and a day with a standardized meal in 15 patients with type 1 diabetes (6 female/9 male; age 31±8 years (mean±SD); BMI 26,5± 2,9 kg/m^2; HbA1c 7,1±0,9%; duration of diabetes 4± 3 years). Venous blood glucose levels were measured continuously by means of the Biostator and in sc interstitial fluid by means of a microdialysis technique. In parallel, venous blood glucose levels were measured discontinuously by means of a laboratory method (reference values) and in capillary blood samples collected at 3 measurement sites (finger, lower arm, and abdomen) by means of three blood glucose meters (one for each site; measured in duplicate). On the first study day blood glucose was clamped at a level of 100 mg/dl for 120 min, 300 mg/dl for 120 min at this level, 50 mg/dl for 60 min, 100 mg/dl for another 120 min, with rapid changes in blood glucose between the plateaus. On the next day, the second study day, slower changes in glycemia were induced by means of a carbohydrate-rich standardized meal (starting BG 90–150 mg/dl).
Results: Venous blood glucose values measured with the lab system were comparable with the continuous recording with the Biostator. In contrast, the capillary measurement results are higher at all three measurement sites. This study shows that ISF glucose levels are higher in comparison to venous blood values during hyperglycemia on both study days but are identical to capillary blood glucose values measured in samples collected at the fingertips. Glucose levels in samples collected at the forearm and abdomen are identical to those collected at the fingertips during stable glycemic levels but run behind during rapid changes in glycemia: max. 30 mg/dl after 60 min.
Conclusion: The previously described AST phenomenon could be reproduced in relatively young patients with Type 1 diabetes. No pronounced AST-like phenomenon could be observed with CGM despite considerable rates of change of glycemia.
Supported by Disetronic/Roche Diagnostics

0969

Comparison between CSII with multiple basal lispro infusion rates and MDI with lispro and glargine

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Background and Aims: To compare the effectiveness of insulin lispro and glargine (MDI-glargine) vs CSII in type 1 diabetic patients in good metabolic control.
Materials and Methods: After a one month run-in period 39 type 1 diabetics (age 38±9 years, duration of diabetes 17±8 years, HbA1c 7.6±0.8%, M±DS), treated with CSII for at least 6 months, were randomly assigned to CSII with lispro or to MDI with lispro and glargine. After 4 months of therapy patients were crossed-over to the alternate treatment. We evaluated HbA1c, blood glucose levels and number of hypoglycaemic episodes. During the last month of each treatment patients underwent 48 hours of continuous glucose monitoring. The satisfaction was evaluated with the DTSQ questionnaire.
Results: HbA1c did not change with both treatments. Compared to CSII, MDI- glargine was associated to higher preprandial glycaemic values (153±71 vs 147±67 mg/dl; P<0.005), higher fasting glycaemic variability (66±15 vs 59±16 mg/dl, P<0.05), higher total daily insulin dose (0.63±0.15 vs 0.54±0.12 UI/Kg, P<0.05). Continuous glucose monitoring profiles in 11 patients (mean duration 34±3 hours) showed a lower glucose exposure with CSII (mean glycaemia 147±10 mg/dl vs 189±11 mg/dl, p<0.02; glycaemia>65 mg/dl and <180 mg/dl 1568±154 vs 929±150 min, p<0.02, glycaemia>180 mg/dl 519±122 vs 1144±205 min, p<0.02). During the night patients using CSII were exposed to glycaemic values>65 mg/dl and <180 mg/dl for a longer period than patients using MDI (371±52 vs 232±48 min, p<0.02, mean glycaemia 135±11

vs 186±12 mg/dl, p<0.02). Patients using CSII had greater satisfaction.
Conclusion: In patients treated with CSII, preprandial glycemia, glycaemic variability and insulin dose were lower and the satisfaction higher. The reduction of glycaemic variability could be important in the prevention of diabetic complications.
Supported by Disetronic Medical Systems AG, Switzerland

0970

Type 1 diabetes. Modalities of external insulin pump treatment initiation in France

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Background and Aims: The efficiency of insulin pump treatment on glycaemic control and patient quality of life has been clearly demonstrated. Thus, the number of insulin pump treated patients should keep on increase in the future. In this context, it is pertinent to describe the medical practices in insulin pump initiation in patients with type 1 diabetes, and to compare them to official guidelines (Alfediam, France).
Materials and Methods: A transectional study was conducted in France in 2004/2005 with 110 diabetologists from 68 centres (general public hospitals: 48%, university hospitals: 35%, private hospitals: 11%, others: 6%). Each participating physician had to select 3 type 1 diabetic patients>15 years old, whose insulin pump treatment was initiated at the inclusion visit or less than 3 months before.
Results: Among the 349 diabetic patients included, 62% were women. Their mean age was 37±14 and the mean duration of diabetes was 15±10 years. Before pump initiation, 73% of the patients were treated by rapid-acting insulin analogues; 54% by long-acting insulin analogues. Severe hypoglycaemia and ketoacidosis had occurred for respectively 17% and 6% of the patients in the six months preceding pump initiation. Before being treated by pump, patients were used to perform 4.3±1.8 capillary blood glucose measurements every day and they reported 3.1±2.6 symptomatic hypoglycaemia episodes per week. Mean number of insulin injections per day was 3.8±0.8 but 24% of the patients had less than 4. When pump was initiated, the mean HbA1c value was 8.44±1.58%. HbA1c was above 8% (guidelines indication) for 56% of the patients, of which 19% were women waiting for pregnancy. HbA1c was>10% for 14% of the patients, <8% for 42%, and <7% for 14%. Two thirds of the patients with HbA1c <8% were waiting for a pregnancy, presenting recurrent severe hypoglycaemia or ketoacidosis, or having irregular lifestyle (guidelines indication). The prescribed treatment consisted in rapid insulin analogs for most of the patients (86%), with 6 home blood glucose measurements or more per day (76%) and an urinary (77%) or capillary (35%) ketone bodies monitoring. The pump initiation was mainly motivated by the uncontrolled status of diabetes (75%) and patient's demand (43%). Therapeutic goals declared by the physicians were to get an HbA1c value below 7% (88% of cases), to improve patient's quality of life (70%), and to prevent diabetes complications (69%).
Conclusion: Practices in insulin pump treatment of type 1 diabetes are mostly consistent with French official guidelines, especially concerning HbA1c values at initiation and therapeutic goals. However, pump initiation is not always preceded by an intensification of insulin treatment using multiple insulin injections. Frequent hypoglycaemia and patient's demand are increasing motivation factors for pump prescribing.

0971

Using insulin pump therapy together with real time continuous glucose monitoring in the therapy of type 1 diabetic patients: the impact on glycaemic excursion - a pilot study

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Background and Aims: to confirm the efficacy of a new therapeutic system - sensor augmented insulin pump Paradigm® RT (Medtronic Inc), which displays updated subcutaneous glucose readings every five minutes and sounds the alarm when levels become over or below previously determined values.
Materials and Methods: 7 patient (type 1 diabetic men, treated with CSII, older Paradigm type). Each patient was continuously monitored for 5 days (CGMS Gold) and was not allowed to see the results. After detailed training the older Paradigm type pump was replaced by Paradigm® RT and each patient used this system for another 5 days. We compared the frequency of symptomatic hypoglycaemias and the total duration of glycaemic excursions over 10 mmol/l and below 3,5 mmol/l between those two 5 day periods (Monday - Friday).

Results: we found a decrease in the average number of symptomatic hypoglycaemias per 5 days using Paradigm® RT: 2,1±0,78 v.s. 3,33±0,81 (ns) in the previous period, an average total duration of hypoglycaemic excursion per 24 hours decrease to 21 minutes v.s. 105 minutes (ns) previously and the duration of hyperglycaemic excursion decrease to 202 minutes v.s. 352 minutes (ns) in the previous period.
Conclusion: the first experience using the Paradigm® RT indicates an important influence on the stabilization of glycaemic levels in type 1 diabetic patients. More patients are to undergo the study to confirm these results definitively.
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0972

Treatment satisfaction and metabolic control in type 2 patients using CSII: results from an international observational study

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Background and Aims: The progressive character of type 2 diabetes with an increasing loss of β-cell function often results in the need for insulin treatment to recover good metabolic control. Intensification of insulin treatment including CSII is also an attractive option for such patients if basal insulin supply needs to be optimised and hypoglycemic episodes shall be minimised. However, few systematic data exist on patients with type 2 diabetes using insulin pumps. We documented data from those patients in routine daily life.
Materials and Methods: A structured questionnaire was distributed to 36,450 patients treated with CSII across Europe and North America to determine patient characteristics, medical data, treatment complications and satisfaction. In total, there were 25 structured questions with additional information on socio-demographic background, insulin pump indication, initiation and follow-up of treatment. We report the analysis of the sub-set of data of patients with type 2 diabetes on CSII.
Results: A 38.4% response rate was achieved with 14,015 questionnaires returned, out of which 729 (5.2%) were patients with type 2 (64% males). They had a mean (±SD) age of 56.4±11.9 years, a BMI of 29.9±5.9 kg/m^2, a duration of diabetes of 17.2±8.9 years, a duration of insulin use of 10.8±7.8 years and were already 3.4±3.2 years on CSII. Theses patients detailed multiple reasons for selecting CSII therapy out of choice of 12 indications: The most common was poor glycemic control with s.c. insulin therapy (76.5%), uncontrolled blood glucose fluctuations (44.4%) and high insulin requirements with syringe/pen therapy (34.4%). Initiation of CSII was carried out either at hospital as in- (44.3%) or out-patient (5.5%), in diabetes specialist's office (46.4%) or in a general practitioner's office (2.9%). The average total daily insulin dose applied was 0.84±0.6 IU/kg body weight whereas 49.3% was given as basal insulin reflecting a close to 50:50 ratio. Patients achieved a HbA1c of 7.0±1.2% (non-standardized), with the majority of patients (67.2%) being below 7.5%. Patients were highly satisfied with CSII therapy. They left their infusion set in place for 2.7±1.2 days.
Conclusion: This international observational study provides real world data about a relatively "unknown" patient group, i.e. patients with type 2 diabetes on CSII. Patients are relatively young and have already a significant duration of diabetes and insulin use before starting insulin pump treatment. They achieve good glycemic control with a 50:50 ratio basal/bolus insulin for the average patients with high treatment satisfaction. Clearly, the evidence for the use of CSII in such patients should be studied in a randomised controlled clinical trial.
Supported by Disetronic Medical Systems AG, Switzerland

0973

Glycaemic control, health status and treatment satisfaction in subjects with diabetes mellitus treated with Continuous IntraPeritoneal Insulin Infusion (CIPII). A single centre experience

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Background and Aims: Continuous intraperitoneal insulin infusion (CIPII) has been in use now for over 20 years. High costs and technical problems have prevented a widespread use, with most intraperitoneal (IP) pumps implanted in France and the USA. In the Netherlands, the Isala Clinics in Zwolle is the centre with the most experience with CIPII. Its use is aimed at improving glycaemic control with less hypoglycaemic events, and thus improving health related quality of life in patients with poorly controlled diabetes despite intensified insulin treatment with multiple subcutaneous injections or an external pump.

The aim of this study is to assess effects of CIPII on glycaemic control, health status and treatment satisfaction in all subjects treated with CIPII within the Isala Clinics.
Material and methods: Retrospective longitudinal analysis of clinical data in 48 patients initiated on CIPII between 1983 and 2005. HbA1c levels at baseline, after one year, and at present assessment or at end of pump use were applicable. Cross-sectional assessment of health status using the Short Form 12 item health survey (SF-12), of well-being using the Well-Being Index (WHO-5) and of treatment satisfaction using the Diabetes Treatment Satisfaction Questionnaire (DTSQ).
Results: 33 of 48 patients were still treated with CIPII at the moment of assessment (fourth quarter 2005). Over 90% returned the questionnaire. 5 patients died whilst on CIPII (4 diabetes related), none died of hypoglycaemia. Reasons for cessation of CIPII were; pump malfunction (3), inadequate glycaemic control (4), kidney-pancreas transplantation (2) and recurrent infections (1). HbA1c (mean± standard deviation) decreased from 9.3± 1.7% to 8.8±1.7% at one year and 8.7±1.6% at long term follow-up (p=0.004 and p=0.001 respectively as compared to baseline). Patients reported less hypoglycaemic events with CIPII. The Physical Component Summary score of the SF-12 was 37.4±12.1, the Mental Component Summary score was 47.2±11.1 (scores range from 0 to 100 with scores in general population standardised to 50±10). Mean score on the WHO-5 was 52.7±28.6 (score ranges from 0 to 100). Thirteen patients scored below the cut-off point of the WHO-5 for further testing for depression. Median treatment satisfaction score was 32 (P^{25}-P^{75}: 28-36) (score ranges from 0 to 36).
Conclusion: Use of CIPII results in improved glycaemic control with less self-reported hypoglycaemic events in patients with poorly controlled diabetes despite intensified subcutaneous insulin treatment. Treatment satisfaction is high, and comparable to that with other treatment modalities. Mental health status and well-being scores are low however, with nearly 1 out of 3 patients in need of further assessment for depression, at least according to WHO-guidelines.

0974

Modelization of glucose tracking performance of IV sensors and insulin pharmacokinetics by IP delivery during standard meals to improve algorithms used in a closed loop system

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Background and Aims: Effective glucose control at meal times represents an important challenge for the clinical development of closed loop insulin delivery systems. The aim of this study was the modelization of glucose tracking by an intravenous (IV) sensor and insulin kinetics by intraperitoneal (IP) delivery from implanted pumps, in view of more effective algorithms used for automated glucose control by a model of implanted artificial pancreas that combines both devices.
Materials and Methods: IV glucose sensor performance and delays associated with glucose sensing and IP insulin delivery were quantified in 6 type 1 diabetic patients (4M/2F, BMI 22.5+1.0 kg/m2 (mean+SEM), duration of diabetes 46.8+1.9 y, age 62.5+1.9 y). IV sensors had been implanted for ~10 months prior to performing experiments and were linked to IP pumps (both Medtronic MiniMed). Following an overnight fast during which insulin delivery was kept constant (0.6+0.1 U/h), subjects received a meal (100 g carbohydrate) and a bolus of insulin (4.7+0.6 U). Blood was sampled every 5 min until completion of the first 30 min after the meal, every 10 min for the remaining 1st hour, every 20 min for the 2nd hour and every 30 min for the 3rd and 4th hour.
Results: Sensor kinetics were described using a first order model described by a transport delay (T_D) and a time constant t: R^2=0.84+0.1, T_D=21.8+6.1 min, t=20.2+6.3 min. Sensor glucose was well correlated with reference and generated an unbiased estimate of the reference (R^2=0.85+0.03, SG=1.02×reference −25.7, slope not different than 1, P<0.05). IP insulin kinetics were well described (R^2=0.84+0.1) using a bi-exponential model with time delays t_1=60.0+8.7 min and t_2=27.2+9.3 min, and insulin clearance (3600+ 759.2 ml/min).
Conclusion: Besides demonstrating accuracy of IV sensors in tracking glycemic excursions ten months after implantation, the proposed models accurately describe the dynamics associated with IV glucose sensing and IP insulin delivery. Integration of these models with algorithms modulating IP insulin delivery according to IV glucose sensing are expected to allow more effective glucose control at meal times in future closed loop trials with our implanted system.

0975

Automated intensive diabetes management based on continuous glucose monitoring.

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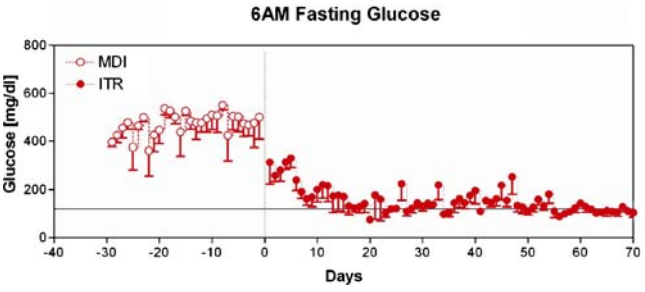
Glucose Sensor R&D, Medtronic MiniMed, Los Angeles, United States.

Background and Aims: Intensive diabetes therapy has been shown to lead to improved glucose control and reduced long-term complications. Nonetheless, adjustments in pump therapy are typically performed empirically. The aim of this study was to demonstrate the feasibility of automating these adjustments using a proportional-integral-derivative (PID) algorithm variation.
Materials and Methods: The proposed algorithm was evaluated over a 12-week period in 4 pancreatectomized canines (1M, 3F 29.9±3.5 kg), fed once per day (6:00 am). During the initial 3 weeks, daytime glucose control was established using Multiple Daily Injection (MDI) therapy. A long acting insulin injection was given with the 6:00 AM meal. Blood samples were drawn daily at 6 AM, 8 AM, 10 AM, 12 PM and 2 PM. The total daily dose (TDD) of insulin was adjusted to achieve good daytime glucose (TDD=46.3±3.3 U). Thereafter, 24-hour glucose control was assessed using a continuous glucose monitoring system (CGMS; Medtronic MiniMed) over six days. At week 4, Continuous Subcutaneous Insulin Infusion (CSII) therapy was initiated (Medtronic MiniMed Paradigm pump). A fixed basal rate (24 hour), Carbohydrate-to-Insulin Ratio (CIR) and Insulin Sensitivity Factor (ISF), were calculated based on the TDD established during MDI therapy (basal=TDD/48, CIR=800/TDD grams covered per U; ISF=2200/TDD). Continuous glucose monitoring was performed throughout the CSII period. Insulin infusion sets were replaced 2 to 3 times per week, and sensors were replaced twice per week. While using CSII, a meal bolus was calculated using the CIR but delivered as a dual wave (50% immediate; and 50% over 3 h). CIR was adapted based on a 3-hour postprandial target of 180 mg/dl. Basal rates were adapted in three-hour blocks (starting at 12 AM) based on target glucose of 120 mg/dl. Correction boluses were given at 10 AM and 12 PM, with the ISF adapted using 2-hour post correction target values. Adaptation was performed using a discrete proportional integral derivative algorithm with changes (recommendations) confirmed by study staff.
Results: Therapy adjustments were made every 4.2± 0.1 days during CSII therapy. Insulin requirement significantly decreased after 10 weeks (46.3±3.3 vs. 31.4±2.6 U, P<0.05). HbA1c decreased during the CSII period, albeit the decrease was not significant (3.9±0.2% vs. 3.7%±0.1, P=NS). Overnight glucose converged to the target glucose concentration approximately 50 days after CSII initiation (see Figure for all 6 AM glucose values; final midnight, 3 AM, and 6 AM glucose levels were 141.0±9.4 mg/dl, 125.1±13.0 mg/dl, 124.0±6.6 mg/dl respectively). Postprandial glucose converged to the 3-hour (9 AM) postprandial setpoint of 180 mg/dl (174.9±7.7 mg/dl) with 12 PM glucose at 157.7±10.1 mg/dl and 3 PM glucose at 115.8± 7.6 mg/dl. Overnight control was stable (129.0±14.2 at 12:00 AM; 130.6±13.1 mg/dl at 9 PM, P=NS).
Conclusion: We conclude that automating pump therapy adjustments is, in principal, a feasible strategy during pump therapy.



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PS 82

Insulin treatment modalities

0976

Metabolic control and hypoglycaemia in 5,891 type 1 diabetic patients on human insulin (HI) or insulin analogues (AI) in routine care in Germany 2005

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Background and Aims: Insulin analogues are increasingly prescribed aiming at improving HbA_{1c}, lowering incidence of hypoglycaemia or increasing quality of life. However, a recent Cochrane review described only minor benefits of short acting analogues in type 1 diabetes. Results of trials with long acting analogues are controversial concerning HbA_{1c} and demonstrated a beneficial effect on hypoglycaemia in some studies. Results from large samples representing the "real life situation" are not available. We analysed 5,891 pat. on human insulin or analogues in the database of the **G**erman **D**iabetes **A**ssociation.
Materials and Methods: The GDA provides a certificate of quality for hospitals and specialised private practices. Candidates have to provide information on staff, teaching programmes and documentation of pat. data. 27 items have to be submitted on>50 pat. with type 1 diab. (and 200 pat.

with type 2 diab. - data not shown). Hospitals collect data of consecutive pat. on admission, in private practice data are taken from a random sample. Data include: age, years since diagnosis, BMI, IU/day, injections/day, HbA_{1c}, severe hypoglycaemia (requiring i.v. glucose or glucagon), use of analogue insulin (yes/no). In 2005 data of 5,891 type 1 diabetic pat. were submitted from 26 private practices (n=1,664) and 92 hospitals (n=4,227). Information about use of short and/or long acting analogues was available in 5,888 pat..

Results: 58% of 5,888 pat. were treated with insulin analogues.

In private practice (n=1661) 63% were on AI. Compared with pat. on HI they had a longer duration of diabetes (16.1 vs 14.8 years)*, more injections per day (4.6 vs 4.4)* and higher HbA_{1c} (7.29 vs 7.12%, DCCT adjusted)*. Not signif. different were: age (43.2 vs 44.7 y), BMI (25.8 vs 26.1 kg/m^2) insulin units per day (47.3 vs 45.5), severe hypoglycaemia (0.06 vs 0.07/pat/year), incidence of comata or severe ketoacidosis (0.01 vs 0.00/pat/year).

In hospitals (n=4,227) on admission 56% of the pat. were treated with AI. Compared with pat. on HI they were younger (42.8 vs 45.5 y)*, they injected more frequently (4.3 vs 4.2/day)*, severe hypoglycaemia had occurred more frequently (0.34 vs 0.24/pat/year)* and the insulin dosage was higher (46.2 vs 43.8 IU/day)*. Not signif. different were: years since diagnosis (16.1 vs 15.4), BMI (25.2 vs 25.1 kg/m^2), HbA_{1c} (8.18 vs 8.23%), coma or severe ketoacidosis (0.12 vs 0.11/pat/year). (*=$p<0.05$).

Conclusion: Preceding admission to hospitals type 1 diab. pat. on HI had experienced significantly less episodes of severe hypoglycaemia compared to treatment with AI. In ambulatory care the use of AI was associated with slightly higher HbA_{1c} compared to pat. on HI. It cannot be excluded that pat. with more severe diabetes problems were treated with AI. The results of our observational data cannot challenge the results of properly designed controlled trials. Nevertheless, in the "real life" situation problems may have occurred, which were not foreseeable by trials. Research is imperative to continuously monitor the various strategies using specific short and long acting analogues and their impact on outcome.

0977

Modelling study comparing complications in type 1 diabetes patients treated with either human or analogue insulin in a basal-bolus regimen

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Background and Aims: Long-term epidemiological studies in type 1 diabetes patients have shown that poor glycaemic control is associated with an increased risk of disease-related complications. In a recent multi-center randomized controlled trial of basal/bolus therapy with insulin detemir plus insulin aspart (analogue) versus neutral protamine Hagedorn (NPH) insulin plus human soluble insulin (human), the analogue regimen was associated with an improved HbA1c profile (0.22%-points lower, p= 0.0004), a reduced risk of hypoglycemic events (RR=0,79, p=0.036) and decreased BMI (−0.3 kg/m^2, $p<0.001$) compared to human insulin after 18 weeks.

The aim of this study was to estimate the time to onset of diabetes related complications for type 1 diabetes patients treated with analogue and human insulin based basal-bolus regimens.

Materials and Methods: A published and validated model of type 1 diabetes, which combines Markov sub-models and Monte Carlo simulation to simulate the progression of the disease, was used project the long-term impact of treatment with analogue and human insulin. Baseline cohort characteristics (mean age 39 years, HbA1c 8.38%-points, BMI 24.9 kg/m^2, duration of diabetes 15 years) and treatment effects observed over the 18-week trial were used in the model to project the time to onset of diabetes-related complications over a 35-year time horizon.

Results: Treatment with analogue insulin delayed the time to onset of any diabetes-related complication by 0.09 years (1.06 versus 0.97 years). Notably, analogue insulin postponed the onset of end-stage renal disease by a mean of 0.34 years (over 4 months) per patient (20.61 versus 20.27 years). Similarly, analogue insulin was projected to delay the mean time to onset of proliferative retinopathy (by 0.48 years), lower extremity amputation (0.32 years), myocardial infarction (0.33 years) and stroke (0.30 years) compared to human insulin.

Conclusion: Improvements in glycemic control, hypoglycemic event rates and BMI associated with analogue insulin compared to human insulin treatment as part of a basal-bolus regimen were projected to delay the onset of diabetes-related complications, which are associated with a substantial clinical and economic burden.

Supported by Novo Nordisk A/S

0978

Basal-bolus therapy with insulin detemir/insulin aspart improves glycaemic control and reduces hypoglycaemia: data from the German cohort of the PREDICTIVE study

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Background and Aims: Basal-bolus (BB) regimens comprising analogue insulins have the potential to offer clinical advantages over BB regimens with human insulins, including improved glycaemic control and reduced risk for hypoglycaemia. The safety and efficacy of insulin detemir, a basal analogue insulin, is currently being evaluated in actual clinical practice in a large, multi-centre, prospective observational study - The Predictable Results and Experience in Diabetes through Intensification and Control to Target: An International Variability Evaluation (PREDICTIVE) study. This report evaluates the safety and efficacy of treatment with an all analogue BB regimen, comprising insulin detemir/insulin aspart in patients switched from a conventional regimen of NPH insulin/ human soluble insulin.
Materials and Methods: Data were compared between baseline and 3 months from 179 patients in the German cohort of the PREDICTIVE study, who switched from NPH insulin/human soluble insulin to insulin detemir/insulin aspart. This cohort was part of the 1811 intensively-treated type 1 patients in the PREDICTIVE study. Key baseline data for the 179 patients were: 54% female; mean age 44.1 years; diabetes duration 17.7 years; HbA_{1c} 8.1%; BMI 25.4 kg/m^2.
Results: Switching BB therapy to insulin detemir/insulin aspart was associated with a marked reduction in the rate of overall hypoglycaemia to 11 events/patient year in the 4 weeks prior to the follow-up visit, as compared with 55 events/patient year in the 4 weeks prior to study start. No major hypoglycaemia was observed at follow-up (reduced from 1.2 events/patient year at study start). The rate of nocturnal hypoglycaemia was also reduced from 18.3 to 1.7 events patient/year. Overall, the proportion of patients reporting all hypoglycaemic episodes fell to 23% from 59% on switching to the all analogue regimen, while the proportion of patients experiencing nocturnal hypoglycaemia was reduced from 39% to 8%. Switching to all analogue BB therapy was also associated with improved glycaemic control: HbA_{1c} was reduced by 0.65% from pre-study values to 7.45%; fasting blood glucose (FBG) was reduced by 39.4 mg/dl. Within-subject FBG variability, calculated as SD of FBG, was also reduced by 13.4 mg/dl. Throughout the observation period, body weight remained stable and unchanged from pre-study values and total insulin dose remained constant (pre-study, 0.68 IU/kg; follow-up 0.71 IU/kg).
Conclusion: The data support that changing BB regimen from human insulin to an all analogue regimen, consisting of insulin detemir and insulin aspart, improves metabolic control and reduces the rate of hypoglycaemia, without increasing body weight, in patients with type 1 diabetes.
Supported by Novo Nordisk A/S

0979

An evaluation of insulin detemir treatment in type 1 and type 2 diabetes: German cohort data from the PREDICTIVE study

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Background and Aims: Observational studies provide an effective method for validating data from clinical trials in actual clinical practice. The Predictable Results and Experience in Diabetes through Intensification and Control to Target: An International Variability Evaluation (PREDICTIVE) study is a large, multi-centre, prospective, observational study in people with type 1 and type 2 diabetes. The aim of the study is to provide an insight into treatment patterns, and the efficacy and safety associated with use of insulin detemir in everyday clinical practice.
Materials and Methods: Data from a cohort of 10,276 patients from Germany participating in the PREDICTIVE study were evaluated before and after (mean follow-up period=14.5 weeks) initiation of insulin detemir. Baseline characteristics of the cohort were: 26% type 1 diabetes, 73% type 2 diabetes, 1% other classification; 52% female. Type 1 diabetes: mean age 45 years, mean duration of diabetes,17 years; type 2 diabetes, mean age 62 years, mean duration of diabetes, 10 years. The primary endpoint of the study was safety. Secondary endpoints included changes in the following parameters from baseline: HbA_{1c}, weight change, fasting blood glucose (FBG) and FBG variability.
Results: During the follow-up period, 28 serious adverse drug reactions (SADRs) were reported. Most SADRs (79%) were hypoglycaemic episodes. In patients with type 1 diabetes, treatment with insulin detemir was associated with a significant reduction in the rate of major hypoglycaemic episodes from 108 events/100 patient years in the 4 weeks before treatment start to 12 events/100 patient years in the 4 weeks prior to follow-up ($p<0.001$). For patients with type 2 diabetes, the rate of major hypoglycaemia was reduced from 30 to 6 events/100 patient years ($p<0.001$). Glycaemic control also improved during the follow-up period with mean HbA_{1c} values reduced by -0.54% and -0.89% from baseline values in patients with type 1 and type 2 diabetes, respectively ($p<0.001$ vs. baseline for both). Values for FBG and within-subject FBG variability were also significantly reduced from baseline at follow-up. FBG was reduced by 33 mg/dl in patients with type 1 diabetes and by 44 mg/dl in patients with type 2 diabetes ($p<0.001$ for both). Within-subject FBG variability (SD of FBG) was reduced by 13 and 8 mg/dl, respectively

(p<0.001 for both). Treatment with insulin detemir was not associated with weight gain in patients with type 1 diabetes. Mean weight reduction of −0.6 kg from baseline (p<0.001) was reported in patients with type 2 dabetes.Insulin detemir was injected once daily by 29% of type 1 and 75% of type 2 diabetes patients.

Conclusions: Data from the German cohort of the PREDICTIVE study support that insulin detemir improves glycaemic control, reduces the incidence of hypoglycaemia, and maintains weight neutrality in clinical practice.

Supported by Novo Nordisk A/S

0980

Predictors of weight and FBG variability in insulin-treated patients: data from the European Cohort* of the PREDICTIVE™ Study

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Background and Aims: Insulin therapy has traditionally been associated with weight gain, which can result in greater insulin resistance, increased cardiovascular risk and poor compliance. Another concern with earlier insulins was the high within-subject variability of action that can lead to an increased risk of hypoglycaemia. Here we examine predictors of bodyweight and within-subject fasting blood glucose (FBG) variability in insulin-treated patients from the European cohort of PREDICTIVE™ - a large multi-national, prospective, observational study.

Materials and Methods: Baseline data collected from 15,919 insulin-treated patients (6,839 [42.96%] type 1, 8,974 [56.37%] type 2 and 106 [0.67%] other classified) from the European cohort were analysed using multi-factorial analysis of covariance (ANCOVA), to assess the association of current bodyweight and within-subject FBG variability (calculated as the standard deviation of FBG) with: age; gender; diabetes duration; pre-study therapy (OAD plus insulin, basal only, premix, bolus only, basal-bolus); incidence of total hypoglycaemia in the 4 weeks prior to study; and baseline values of HbA_{1c}, mean FBG, FBG variability (in weight model only), BMI (in FBG variability model only), total number of injections and total insulin dosage per kg bodyweight (IU/kg).

Results: In both type 1 and type 2 diabetes patients, weight showed a significant positive association with gender (male>female) and total insulin dosage (IU/kg; P<0.0001 for all). Weight was also positively associated with FBG variability (P<0.0001) in type 1 patients, and age (P<0.0001), total number of injections (P<0.0001) and pre-study therapy (P<0.01) in type 2 patients. Neither weight nor FBG variability were associated with the incidence of total hypoglycaemia. The proportion of patients with type 1 diabetes using once versus twice-daily basal insulin in their treatment regimen was 59.1% vs 40.9% respectively; in type 2 patients this proportion was 75.8% vs 24.2%. There was no difference in the FBG variability between the once- and twice-daily basal insulin users in either type of diabetes.

Conclusion: The analysis shows that, except for gender and total insulin dosage, weight was associated with different variables in type 1 versus type 2 patients. In type 1 diabetes there was a strong association with FBG variability. In type 2 diabetes there was an association with pre-study treatment regimen. Three-quarters of insulin-treated type 2 patients received once-daily injection of basal insulin.

*Austria, Czech Rep., Denmark, Finland, Germany, Ireland, Israel, Netherlands, Sweden, Turkey and UK

Supported by Novo Nordisk A/S

0981

Glucose control among patients using and not using insulin: a systematic review of HbA_{1c} at entry in 93 clinical trials, 2000–2004

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Background and Aims: The importance of good glycaemic control has been well-established by landmark clinical trials, such as the DCCT and the United Kingdom Prospective Diabetes Study. Subsequent studies continue to document the efficacy of more intensive therapeutic regimens, including the benefits of early and aggressive use of insulin. Nevertheless, clinicians are intuitively aware that many patients continue to have suboptimal glycaemic control. This study used existing data from published clinical trials to assess glycaemic control at trial entry across a broad spectrum of patients. We also compared glycaemic control among those using and not using insulin.

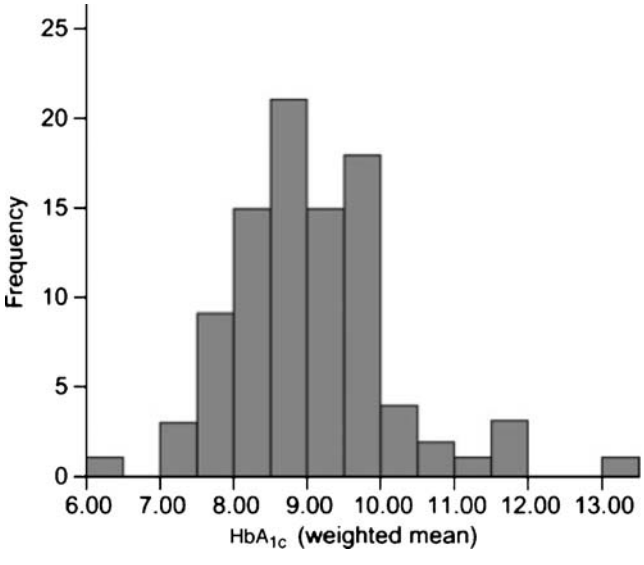
Materials and Methods: We searched MEDLINE using Medical Subject Headings (MeSH) terms ‘type 2 diabetes’, ‘clinical trial’, and ‘insulin’ for the period of January 1, 2000-January 10, 2005, to identify papers reporting data on baseline HbA_{1c} in people entering clinical trials. We also scanned the reference lists of any studies retrieved to complement the original search. So as not to artificially inflate the mean HbA_{1c} value at trial entry, we excluded

studies indicating either that enrolment was limited to patients with HbA_{1c}≥8.5%, or that HbA_{1c} values were determined after a washout period between oral antidiabetic therapy and starting insulin. An overall HbA_{1c} was calculated by weighting the mean HbA_{1c} from each trial by the number of patients enrolled.

Results: A total of 121 relevant publications were identified; 28 were excluded because insulin naïve and insulin-treated patients were not reported separately, the trial was limited to patients with $HbA_{1c} \geq 8.5\%$, HbA_{1c} was obtained after washout period, or baseline HbA_{1c} was not reported. This left 93 trials involving 15,833 people with type 2 diabetes in the final analysis. The weighted mean HbA_{1c} at baseline was 9.07% for 7684 insulin-naïve patients and 8.51% for 8149 insulin-treated patients. The distribution of mean HbA_{1c} for these trials is shown in the figure.

Conclusion: Data from a large sample of clinical trials in diverse groups of patients with type 2 diabetes demonstrate that mean HbA_{1c} values of groups of patients at trial entry frequently exceed recommended glycaemic targets by a clinically significant margin (1.5–2.0 absolute percentage points). Since mean HbA_{1c} values reflect a distribution of individual patient values above and below that value, these results indicate that the discrepancy between target values and glycaemic control in clinical reality was substantially worse for many patients. Although it was not unexpected that, at the time of enrolment in a clinical trial, patients would have suboptimal glycaemic control, it was surprising that the weighted mean HbA_{1c} for patients taking insulin was only about 0.5% better than insulin-naïve-patients. This suggests that either titration of insulin was insufficient, or adherence to prescribed therapy was poor.

Figure: Distribution of mean HbA_{1c}



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0982

Failure of insulin monotherapy in patients with type 2 diabetes: a population-based study

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Background and Aims: Clinical trials have shown that insulin can be used to help patients with type 2 diabetes reach recommended glycaemic targets. Theoretically, insulin therapy should have comparable efficacy when initiated outside of the structured environment of a clinical trial. Despite the prevalence of its use, the extent to which insulin users in the general population reach these glycaemic targets is largely unknown. In the UK and Germany, we assessed glycaemic control in a clinical setting via a population-based analysis of last recorded HbA_{1c} levels, using data from adult patients who were prescribed insulin and who had not been prescribed oral antidiabetic drugs (OADs) within the previous 3 months.

Materials and Methods: Data were obtained from IMS Health, Inc, who collected longitudinal population-based data for up to 14 years from computerised primary care practices in the EU. These data were sampled from 6 million patient records from 1045 physicians in 983 practices in Germany and 3.6 million patient records from 630 physicians in 200 practices in the UK.

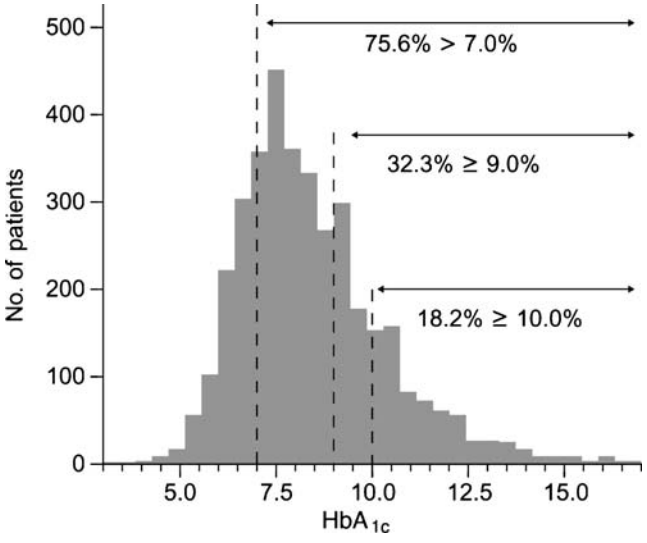
Results: Overall mean and median HbA_{1c} were 8.42% and 8.10%, respectively, for 3658 patients with type 2 diabetes (Figure). Insulin has the potential to enable patients to achieve targets, and given the difficulty of persuading patients to initiate insulin, it is discouraging that many insulin users remained above recommended glycaemic targets. About one-third (32.3%) had HbA_{1c}≥9.0% and almost one-fifth (18.2%) had HbA_{1c}≥10.0%. Similar proportions of patients could be categorised as poorly controlled in both countries (ie, 30.1% HbA_{1c}≥9.0% and 18.6% HbA_{1c}≥10.0% from Germany, and 34.0% HbA_{1c}≥9.0% and 17.9% HbA_{1c}≥10.0% from the UK), suggesting a robustness to the findings.

Having excluded from the sample patients prescribed OADs in the previous 3 months, it was not clear how well the analysis reflected the level of control of people using insulin plus OADs. Although it might be logical to assume that patients using OADs may have better control, ultimately the direction and magnitude of any bias resulting from omission of OAD users is unknown. Comparison of the results from the UK and Germany must be made with caution, as the proportion of laboratories standardising their results to the DCCT measurements (i.e., those who are DCCT-aligned) are not the same. Most labs in the UK are

DCCT aligned, whereas this is not the case in Germany, where at least 4 different methods are used. Thus, normal ranges may differ somewhat.

Conclusions: These data suggest that glycaemic control may be suboptimal (HbA_{1c}>7.0%) for up to three-quarters of patients on insulin monotherapy. Identifying and overcoming obstacles preventing optimal insulin therapy, including adequate titration, is essential if the gap between recommended glycaemic targets and control in the clinical setting is to be closed.

Figure. Distribution of HbA_{1c} for 3658 patients with type 2 diabetes in Germany (n=1536) and the UK (n=2122) prescribed insulin, 2004



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PS 83

Insulin trials in type 2 diabetes

0983

Effect of biphasic insulin aspart 30/70 (BIAsp30) in combination with metformin on glycaemic control in subjects with type 2 diabetes not optimally controlled on oral antidiabetic agents

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Background and Aims: Biphasic insulin aspart 30/70 (BIAsp30) is a new premixed insulin analogue comprising 30% soluble and 70% protamine-crystallised insulin aspart. Addition or substitution with twice-daily premixed insulin analogue/human insulin is a common treatment approach in subjects with type 2 diabetes (T2DM) inadequately controlled on oral antidiabetic agents (OADs). There is growing evidence that addition of once-daily BIAsp30 to OADs provides significant clinical benefit to subjects with T2DM. This trial was designed to evaluate whether once-daily BIAsp30 at dinner in combination with metformin (BIAsp30+Met) provides glycaemic control that is at least as efficacious as twice-daily BIAsp30 alone (BIAsp30).

Materials and Methods: This 24-week, multicentre, open-labelled, parallel, randomised (1:1) comparison trial included 140 subjects with T2DM (BIAsp30+Met vs BIAsp30: mean T2DM duration 8.2 vs 9.7 years, HbA_{1c} 10.1 vs 10.2%, BMI 28.8 vs 29.5 kg/m^2) in South Africa inadequately controlled on one or two OADs. At randomisation, OADs were discontinued and subjects received BIAsp30+Met or BIAsp30. Efficacy was assessed by HbA_{1c}, prandial plasma glucose (PG) increment and plasma lipids. HbA_{1c} at 24 weeks was analysed using ANOVA with adjustment to baseline, and comparison between treatments was performed using a predefined non-inferiority criterion of 0.6%. Safety was evaluated by incidence of hypoglycaemic episodes and adverse events.

Results: After 24 weeks of treatment, glycaemic control was improved in both treatment groups: HbA_{1c} was reduced by 1.90±1.44% in the BIAsp30+Met group and 1.62±1.74% in the BIAsp30 group and non-inferiority between treatments was confirmed (diff −0.31%; 95% CI [−0.752; 0.129], p=0.164). Consistent with the trend in HbA_{1c}, improvement was observed in change from baseline in average prandial (breakfast, lunch and dinner) PG profiles in both the BIAsp30+Met group (−1.18± 2.47 mmol/L, p=0.0183) and BIAsp30 group (−0.86± 2.41 mmol/L, p=0.0054). Improvement in glycaemic control was associated with slight weight changes from baseline in the BIAsp30+Met group (0.50±3.33 kg) and BIAsp30 group (3.69±4.91 kg). Total cholesterol decreased by 0.12±1.02 mmol/L in the BIAsp30+Met group and increased by 0.21±0.88 mmol/L in the BIAsp30 group (diff −0.34 mmol/L; 95% CI [−0.678; −0.004]). Other parameters (LDL-, HDL-cholesterol and triglycerides) were clinically comparable in both treatments. Mean total daily dose of BIAsp30 was 0.36 U/kg in the BIAsp30+Met group (with daily metformin dose of 2324 mg) and 0.65 U/kg in the BIAsp30 group. A total of 94 hypoglycaemic episodes were reported by 28 subjects on BIAsp30+Met and 147 hypoglycaemic episodes by 30 subjects on BIAsp30, of which all were minor or symptomatic, except for one major event in the BIAsp30+Met group. Adverse events were similar between treatments.

Conclusion: In subjects with T2DM inadequately controlled on OADs, addition of once-daily BIAsp30 to metformin therapy or substitution with twice-daily BIAsp30 offer significant clinical benefit. The barrier to insulin initiation can be overcome with BIAsp30 once-daily with metformin, which offers a convenient, safe and effective approach to treat T2DM.
Supported by Novo Nordisk

0984

Effect of BIAsp 30 (Biphasic insulin aspart 30) in combination with oral hypoglycaemic agents on glycaemic regulation in non-obese patients with type 2 diabetes

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Background and Aims: Biphasic insulin aspart (BIAsp30, 30% soluble and 70% protaminated insulin aspart) is a premixed insulin analogue targeting both postprandial and basal glucose regulation. The objective was in a randomized double masked trial to study the effect of BIAsp30 in combination with oral hypoglycaemic agents (OHA) on glycaemic regulation in *non-obese* patients with type 2-diabetes (T2DM). A dose titration algorithm with increasing numbers of insulin injections was applied to reach the glycaemic target.
Materials and Methods: 102 non-obese (BMI≤27 kg/m^2) patients with T2DM and poor glycaemic regulation (HbA_{1c}≥6.5%; normal range: 4.1–6.4%) were included after a 4 months run-in on combination therapy with repaglinide plus metformin. At baseline (0 months) patients were randomized to either repaglinide 6 mg daily or metformin 2 g daily (double-masked), both in combination with BIAsp30 (once daily, pre-dinner) for 12 months. Patients self-adjusted insulin dose according to an algorithm aiming for fasting plasma-glucose of 4.0–6.0 mmol/l and HbA_{1c} <6.5%. Patients intensified to 2 or 3 daily injections after 3, 6 or 9 months if target glycaemic goals were not reached, or earlier if hypoglycaemia prevented an increase in insulin dose. Predefined endpoints were assessed at 0, 3, 6, 9 and 12 months. Concomitant OHA therapies remain double-masked until June 2006, and the extent to which combination of insulin with metformin or repaglinide treatment influenced HbA_{1c} will be determined immediately thereafter.
Results: HbA_{1c} (mean±SD) was significantly reduced (7.56%±0.91; 7.02%±0.67; 6.96%±0.67 and 6.79%±0.66) at 3, 6, 9 and 12 months, respectively, versus baseline (8.10±1.40), p<0.001 for all comparisons versus baseline. HbA_{1c} decreased significantly between all successive visits (p<0.001), except from 6 until 9 months where HbA_{1c} remained stable. Target (HbA_{1c} <6.5%) was reached in 11% (n=11), 20% (n=20), 23% (n=23) and 35% (n=36) of all patients after 3, 6, 9 and 12 months, respectively. During the 12 months intervention period 43% (n=44) of all patients obtained at least one measurement of HbA_{1c} <6.5% and 25% (n=25) of all patients obtained at least two measurements of HbA_{1c} <6.5%, respectively. Among patients with HbA_{1c}≥7.0% at baseline (n=77), 64% (n=49) achieved HbA_{1c} <7.0% after 12 months. After 3 months 10% (n=10) of all patients achieved HbA_{1c} <6.5% with once daily BIAsp30 injection compared to 6% (n=6) of all patients after 12 months. The percentage of patients who received 1, 2 and 3 daily injections of BIAsp30 was 14% (n=14), 42% (n=41) and 43% (n=42), respectively after 12 months. Median (range) dose of BIAsp30 was 30 U (6–108) after 12 months. During the 12 months intervention period only 5% (n=5) of all patients dropped out.
Conclusion: BIAsp30 in combination with OHA results in long-lasting near-optimal glycaemic regulation in non-obese patients with T2DM uncontrolled by OHA combination-therapy. A treatment algorithm using increasing number of insulin injections and patient self-adjustment of BIAsp30 dose can obtain glycaemic targets in many patients while maintaining a high level of adherence to therapy. BIAsp30 in combination with OHA can be initiated once daily pre-dinner, but most non-obese patients with T2DM need more than one daily injection to reach and maintain glycaemic regulation within targets after one year.
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0985

Insulin glargine-based therapy improves glycaemic control in patients with type 2 diabetes suboptimally controlled on premixed insulin therapies

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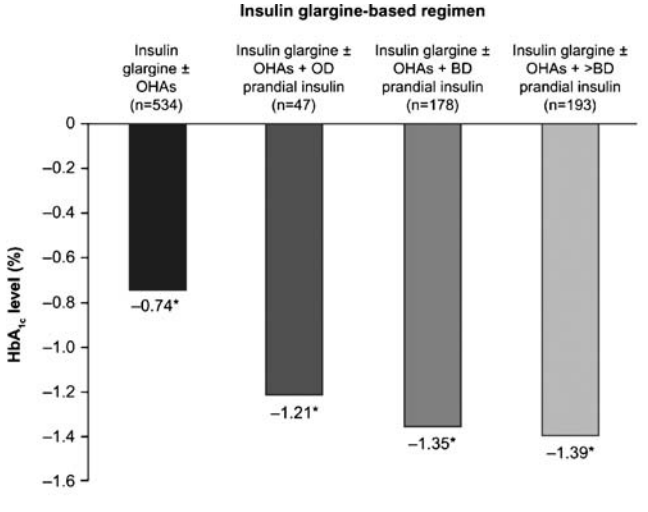
Background and Aims: The AT.LANTUS study compared insulin glargine initiation and titration via one of two algorithms in poorly controlled type 2 diabetes (T2DM) patients. We report results of a subanalysis of patients transferring from premixed insulin±oral hypoglycaemic agents (OHAs) to a once-daily (OD) glargine-based regimen (±OHAs).

Materials and Methods: In a multicentre (n=611), multinational (n=59), open-label, 24-week, randomized trial in 4961 suboptimally controlled T2DM patients, glargine dose was titrated to the target fasting blood glucose (FBG) 5.5 mmol/L (≤100 mg/dL) according to Algorithm 1 (clinic driven) or Algorithm 2 (patient driven). The primary outcome measure was incidence of severe hypoglycaemia.
Results: Patients previously on premixed insulin (mainly two injections/day in all subgroups) at baseline (n=952) transferred to OD glargine±OHAs without (n=534; 56.1%) or with prandial insulin OD (n=47; 4.9%), twice-daily (BD; n=178; 18.7%) or>BD (n=193; 20.3%). Baseline HbA_{1c} was 8.87±1.27%, 8.94±1.31%, 9.11±1.23%, 9.15±1.14%, respectively. When transferred onto glargine, the incidence of severe hypoglycaemia was low (2.71, 0.00, 3.47, 1.05 events/patient-century, respectively), with significant decreases in HbA_{1c} (Figure; p<0.001 for baseline-endpoint change; p=n/s for between algorithms) and FBG (−3.1, −3.3, −3.2, −3.8 mmol/L [−55.2, −58.7, −57.5, −67.6 mg/dL], respectively; p<0.001 for baseline-endpoint change; p=n/s for between algorithms) and small weight changes (+0.4,+1.4,+1.7,+1.5 kg, respectively; p≤0.005).
Conclusion: This study showed that both physician-led and patient-led treatment algorithms were associated with similar improvements in glycaemic control (FBG and HbA_{1c}) within each of the four sub-groups. Furthermore, T2DM patients suboptimally controlled on premixed insulin-based regimens can safely and effectively transfer to a glargine-based regimen±the addition of prandial insulin. Prandial insulin confers added benefit over glargine+OADs alone, with the biggest improvement seen after addition of the first prandial dose. The concept of basal plus one injection of prandial insulin warrents formal evaluation.



*p <0.001 from baseline to endpoint; OHA=oral antihyperglycemic agent; OD=once daily; BD=twice daily

Supported by sanofi-aventis

0986

Glycaemia optimization treatment (GOT): Glycaemic control and rate of severe hypoglycaemia for 5 different dosing algorithms of insulin glargine (GLAR) in patients with type 2 diabetes mellitus (T2DM)

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Background and Aims: This 24-week, randomized, open-label study compared the rate of severe hypoglycemic events (primary endpoint) for 5 dosing algorithms of GLAR, as well as the proportion of patients reaching A1C levels <7.0%.
Materials and Methods: The intent-to-treat population included 4824 patients with T2DM and A1C≥7.0%, who were not currently on insulin therapy. Patients continued prior oral agents (except thiazolidinediones, which were discontinued) and began GLAR 10 U/d, which was actively titrated weekly according to the algorithm group (defined by fasting blood glucose [FBG] goals of 80, 90, 100, 110, or 120 mg/dL, respectively).
Results: Demographics (median age 56 y, 48% male, 70% white, mean diabetes duration 8.3 y, baseline characteristics (mean A1C 9.3%, BMI 34.7 kg/m^2) and study completion rates (88.4%–90.5%) were similar among groups. A1C values improved in all groups in a linear progression from 1.7% to 1.9% below baseline. Despite low overall hypoglycemic rates, severe hypoglycemia was associated with increasingly aggressive dose titration. More aggressive FBG targets required higher doses of GLAR, resulting in greater improvements in A1C and a higher proportion of patients achieving A1C <7.0%. The number of patients achieving A1C <7.0% at the expense of a severe hypoglycemic event was lower with less aggressive dose titration. However, within each dosing algorithm, the risk of severe hypoglycemia was not higher among patients who achieved target A1C <7.0%.
Conclusion: Comparison of results in the different target groups shows that targeting FBG of 110 mg/dL yielded the best balance between resulting A1C levels and the risk for hypoglycemia. Aiming at this goal should allow more patients to achieve target A1C (<7.0%) without encountering more hypoglycemia.

Treatment Group (Goal FBG)*	GLAR Daily Dose, IU Mean (SD), Endpoint	A1C, % Mean (SD), Endpoint	Proportion of Patients With A1C <7.0%, %	Observed Rate of Severe Hypoglycemia†	Modeled‡ Rate of Severe Hypoglycemia (SE)†	A1C <7.0% Observed Rate of Severe Hypoglycemia†	A1C ≥7.0% Observed Rate of Severe Hypoglycemia†
120 mg/Dl N=952	59.2 (36.6)	7.58 (1.1)	31.5 298/945	0.02	0.02 (0.007) (0.01, 0.04)	0.02	0.02
110 mg/dL N=974	62.2 (37.3)	7.52 (1.1)	32.2 812/969	0.07	0.03 (0.008) (0.02, 0.05)	0.02	0.08§
100 mg/dL N=973	69.6 (41.0)	7.41 (1.1)	37.5 363/967	0.04	0.05 (0.009) (0.04, 0.07)	0.04	0.05
90 mg/dL N=950	74.9 (50.3)	7.36 (1.1)	41.1 388/943	0.10	0.08 (0.012) (0.06, 0.11)	0.08	0.12
80 mg/dL N=975	78.1 (42.9)	7.32 (1.2)	44.3 428/966	0.15	0.13 (0.023) (0.09, 0.18)	0.11	0.19

*Intent-to-treat subjects.

†Rate=events/patient-year. Severe hypoglycemia defined as self-monitored blood glucose <36 mg/dL or patient required assistance and responded promptly to treatment with glucagon or glucose.

‡ Means based on unadjusted data using a Poisson regression model.

§A1C <7.0% rate differs significantly from A1C≥7.0% rate (P<0.05)

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0987

Glycaemic control and safety of insulin glargine (GLAR) in elderly patients with type 2 diabetes mellitus (T2DM): The GOAL A1C Study

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Background and Aims: GOAL A1C (*G*lycemic *O*ptimization with *A*lgorithms and *L*abs *A*t Po*I*nt of *C*are), the primary results of which were previously reported, assessed the impact of active vs usual monitoring of insulin titration and point-of-care vs lab A1C testing on glycemic control. This 24-wk multicenter (2164 US sites) open-label study conducted mainly by primary care physicians evaluated the addition of GLAR in 7893 T2DM patients uncontrolled on oral agents (A1C>7.0%).

Material and Methods: To assess the safety and efficacy of GLAR in the elderly, this analysis used all subjects from the primary efficacy cohort (all patients were treated with GLAR; n=5721), and subdivided patients into those aged <65, 65–74, and>75 years.

Results: Among patients with comparable baseline FPG, older patients (65–74 years and>75 years) achieved significantly greater FPG reductions by week 24. However, among patients with comparable baseline A1C, older patients achieved smaller A1C reductions by week 24. Lower FPG, hence, lower glargine doses may have prevented further reductions in A1C in the older patients. There were significant differences among the 3 age groups in the unadjusted rates of hypoglycemia; however, these differences were explained primarily by differences in BMI. Weight gain was greatest in patients <65 y and lowest in patients>75 y, even after controlling for baseline factors.

Conclusion: Adding GLAR to oral therapy improves glycemic control with low overall rates of hypoglycemia in patients 65–75 and>75 with T2DM in primary care settings.

Study Population Age Subgroup Unadjusted Means

		Age <65 y (n=4251)	Age 65–74 y (n=1086)	Age≥75 y (n=384)
GLAR daily dose, U	Week 24, Mean (SE)[1]	58.3 (0.5)	45.3 (1.1)	37.1 (2.1)
FPG, mg/dL	Baseline, mean (SE)	215.0 (1.1)	200.2 (1.8)	197.9 (3.5)
	Week 24, mean (SE)	127.1 (0.7)	115.8 (1.0)	110.8 (1.4)
	Δ, mean (SE)[2]	−87.9 (1.2)	−84.4 (2.1)	−87.1 (3.8)
A1C, %	Baseline, mean (SE)	9.00 (0.02)	8.56 (0.04)	8.45 (0.07)
	Week 24, mean (SE)	7.46 (0.02)	7.43 (0.03)	7.44(0.05)
	Δ, mean (SE)[3]	−1.54 (0.02)	−1.13(0.04)	−1.01(0.07)
Hypoglycemia (events/patient-years)	SMBG <70 mg/dL (SE)[4]	4.5 (0.12)	6.4 (0.4)	8.1 (1.0)
	SMBG <50 mg/dL (SE)[4]	0.55 (0.04)	0.59 (0.07)	0.68 (0.15)
	Severe (SE)[4]	0.08 (0.01)	0.16 (0.05)	0.21 (0.08)

FPG=fasting plasma glucose; SE=standard error

[1]All pairwise comparisons statistically significant ($p<0.001$, adjusting for study arm, BMI, use of sulfonylurea, metformin, and TZD discontinuation).

[2] Percent reduction from baseline was largest in the elderly groups ($p<0.0005<65$ vs 65–74 y; $p<0.0005<65$ vs >75 y, adjusting for study arm, BMI, baseline FPG, use of sulfonylurea, metformin, and TZD discontinuation).

[3] $p<0.01<65$ vs 65–74 y and <65 vs >75 y, adjusting for study arm, BMI, baseline A1C, use of sulfonylurea, metformin, and TZD discontinuation.

[4] No statistically significant differences found between age groups after adjusting for study arm, BMI, gender, use of sulfonylurea, metformin, and TZD discontinuation ($p>0.05$ for all pairwise comparisons).

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0988

Comparison of insulin lispro mid mixture (MM) plus metformin (Met) with glargine (G) plus met on HbA_{1C} (A1C) and blood glucose (BG) profiles in patients with type 2 diabetes (T2D)

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Background and Aims: Various insulin regimens used to attain improved glycemia in people with T2D, including basal+prandial insulin and basal insulin only, may differ in their outcomes. We compared the effects of MM (50% lispro, 50% NPL [prandial+basal])+Met with G (basal only) +Met on A1C and mean daily and pre-/post-meal BG levels and their excursions in patients with T2D previously treated with oral antihyperglycemic medications and 0–2× daily insulin in a prospective, multinational, open-label, randomized, parallel group study.

Materials and Methods: After the 6-wk lead-in period during which all patients received insulin lispro low mixture (25% lispro, 75% NPL [LM]) bid+Met bid (1000–2000 mg/day), patients (n=315) were randomized to MM (ac meal tid)+Met or G (hs)+Met and treated for 24 wks. Insulins were titrated to achieve fasting [F] BG <6.7 mM in both groups, and the MM group also targeted 2-hr postprandial (pp) BG <8.0 mM. Patients on MM unable to reach FBG target could switch to LM before dinner. Patients measured 7-point BG profiles 3× during the 2 wks before each monthly visit. Chi-square and ANCOVA models (treatment and country as a fixed effect and baseline as a covariate) were used to compare treatment differences.

Results: At baseline, both groups had similar mean A1C (7.8% vs 7.8%), age, sex, BMI, T2D duration, pre-/post-meal BG levels, and mean amplitude of glucose excursion (M) value. One-third (34%) of MM patients

switched to LM before dinner. End-point results (mean±SD) were:

	MM+Met	G+Met	p-value
	(n=157)	(n=158)	
A1C (%), 24 wk	7.1±0.9	7.5±1.0	<0.0001
A1C (%), change at 24 wk	−0.7±0.9	−0.4±0.9	<0.0001
A1C ≤7.0 (n[%]), 24 wk	85 (56.3)	58 (39.7)	0.005
Daily insulin dose (u/kg)	0.7±0.3	0.6±0.3	<0.0001
Overall hypoglycemia (episodes/patient/30 d)	0.7±1.7	0.3±0.8	0.02
Nocturnal hypoglycemia (episodes/patient/30 d)	0.2±0.9	0.2±0.6	0.62
Daytime (non-nocturnal) hypoglycemia (episodes/patient/30 d)	0.5±1.2	0.1±0.4	0.002
Severe hypoglycemia (n[%])	4 (2.5)	4 (2.5)	1.00
BG levels (mM)			
Daily 7-point BG	8.2±1.3	8.7±2.0	0.002
Fasting	8.1±1.8	6.5±1.6	<0.0001
Pre-lunch	7.4±1.9	7.9±2.1	0.03
Pre-supper	8.3±2.0	8.9±2.8	0.04
3 AM	7.6±1.9	7.7±2.4	0.25
2-hr pp BG (mM)			
Breakfast	8.7±2.2	9.2±2.6	0.03
Lunch	8.4±1.9	9.8±2.6	<0.0001
Supper	8.7±2.2	10.7±3.2	<0.0001
Change from baseline: 2-hr pp BG excursion (mM)			
Daily	−1.0±1.6	0.6±1.6	<0.0001
Breakfast	−1.1±2.7	0.8±2.7	<0.0001
Lunch	−1.4±2.8	−0.4±3.0	<0.0001
Supper	−0.4±2.8	1.3±3.0	0.005
M (intraday variability in BG)	17.3±13.8	25.1±24.8	0.0004
Change in M from baseline	−13.6±21.7	−7.0±26.9	0.0004

Conclusion: Premeal MM+Met, compared with G+Met, resulted in better glycemic control in many ways including lower A1C, lower mean daily and pre- (lunch & supper only) and post-meal BG levels and their excursions, similar 3 AM BG, and lower M value, despite higher FBG levels. More patients attained A1C ≤7% in the MM+Met group. Nocturnal and severe hypoglycemia were similar in both groups, though overall hypoglycemia (mild) was higher in the MM+Met group due to a higher rate of mild hypoglycemia during the day. A pre-meal MM+Met regimen is clinically useful for glycemic management of T2D.

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0989

Postprandial increase in hs-CRP in Patients with type 2 diabetes (T2D): comparison of lispro mid mixture (MM) plus metformin (Met) with glargine (G) plus met

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Background and Aims: Inflammatory markers, specifically hs-CRP, are reported to increase after a meal. In T2D the timing and postprandial (pp) change in insulin and/or blood glucose (BG) may affect pp changes in hs-CRP. We compared the effects of basal insulin G with prandial+ basal insulin MM (50% lispro, 50% NPL), both plus Met, on post-breakfast BG, serum insulin, and hs-CRP in patients (pts) with T2D before and after 24 wks of treatment in a prospective, multinational, open-label, randomized, parallel group (parent) study.

Materials and Methods: After a 6-wk lead-in period of lispro low mixture (25% lispro, 75% NPL) bid+Met bid (1000–2000 mg/day), pts were randomized to G (hs)+Met or MM (ac meal tid)+Met. Both groups titrated insulins to achieve FBG <6.7 mM and also 2-hr pp BG <8.0 mM in the MM+Met group. A subgroup of US patients [G+Met, n=27; MM+Met, n=29] participated in the test meal. The night before, pts ate their supper, took their assigned insulin, and fasted overnight. Met was taken 30 min and MM was injected 5 min before a standardized McDonald's breakfast [fat 39 g, CHO 78 g, protein 24 g] followed by sampling for 8 hrs. Change from 0-hr value was used for within- and between-treatment comparisons. ANCOVA models included treatment as a fixed effect and change from 0 hr from the pre-randomization visit as a baseline covariate for all corresponding blood draw timepoints.

Results: Pre-randomization, the changes in BG, insulin, and hs-CRP levels before and after breakfast were similar

in both groups. At endpoint mean levels of BG, insulin, and hs-CRP were:

Time (hrs)	BG [mM]		Insulin [pM]		hs-CRP [ng/mL]	
	MM+Met	G+Met	MM+Met	G+Met	MM+Met	G+Met
0	6.9	5.8	163	156	1947	1797
1	9.1 [a]*	9.5*	440[a]*	244*	2856*	2979*
2	8.3 [a]*	9.6*	411[a]*	255*	2635 [a]*	3175*
3	7.1[a]	9.0*	329*	271*	2381 [a]*	3402*
4	5.9 [a]*	7.7*	243*	222*	1953 [a]	2735*
6	5.3 [a]*	6.0	164	173	1738	2070
8	5.5 [a]*	5.3	141	144	1595	1898

[a] Between groups changes from 0-hr significantly different (p-value <0.05)

* Within group significantly different from 0-hr

Conclusions: In T2D postprandial glycemia is associated with a significant increase in hs-CRP which depends on insulin therapy. At end-point, with MM+Met, peak hs-CRP occurred at 1 hr concomitant with peak BG and insulin; with G+Met, peak hs-CRP occurred later at 3 hr with peak insulin. Compared with the pre-breakfast (0-hr) value, an increase in hs-CRP post-breakfast was seen, with levels increasing 1–4 hrs for the G+Met group versus 1–3 hrs for the MM+Met group. Postprandially, for pts on G+Met vs those on MM+Met, hs-CRP were higher at 2–4 hours and BG were higher at 1–6 hrs and these elevations were associated with lower insulin at 1–2 hrs after breakfast. Whether these differences in this surrogate CV risk marker are associated with decreased CV disease risk is not known.
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0990

The effects of insulin and OHA therapy on beta cell function in newly diagnosed type 2 diabetes mellitus

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Background and Aims: To compare the effect of insulin therapy and OHA therapy on plasma glucose and beta cell function in newly diagnosed type 2 diabetic patients.
Materials and Methods: sixty newly diagnosed type 2 diabetic patients with FPG >11.1 mmol/l were randomized into two groups. The subjects in insulin group received intensive or regular insulin treatment. The subjects in OHA group received one sulfonylurea and metformin treatment. All the subjects keep the therapy for three months after fasting plasma glucose(FPG) below the 6 mmol/l. The two group have intravenous glucose tolerance test (IVGTT) before and after treatment. Fasting plasma glucose, 2 hours postprandial glucose (2 hPG), glycosylated hemoglobin A1c (GHbA1C),fasting plasma insulin(Fin), insulin and C peptide were measured. Compare the mean area under the curve (AUC0-10,AUC10-60) of insulin and C peptide, the insulin peak, the increment of the insulin peak (ΔINS), the ratio of total amount insulin to total amount glucose, the ratio of ΔINS/ΔG, Homaβand Homa IR.
Results: Both patients in two groups were achieved the excellent control of FPG, 2hPG and GHbA1C ($P<0.01$, $P<0.05$),but no significant differents between the groups. The time for two groups achieved FPG below 5.6 mmol/l was 5.57±2.3 days and 7.85±1.34 days($P<0.05$). The AUCI0-10 of two group increased 98 percent and 74 percent respectively, AUCI10-60 increased 70 percent and 34 percent respectively the insulin peak increased 150 percent and 86 percent respectively. The increment of the insulin peak(ΔINS), the ratio of total amount insulin to total amount glucose,the ratio of ΔINS/ΔG, AUC0-10 of C peptide were all increased in two group after treatment, but only the insulin group were significantly increased ($P<0.01$ or $P<0.05$).Moreover,there were more significantly improvement of AUCI0-10, the insulin peak,the increment of the insulin peak(ΔINS) in insulin group than OHA group. After treatment the Homaβ were significantly increased and Homa IR were decreased,but on difference between the group. The increase of Homaβ and decrease of Homa IR were positively correlated with the decrease of plasma glucose.
Conclusion: Both insulin treatment and OHA treatment can effectively control the glycemic in newly diagnosed type 2 diabetic patients with high fasting plasma glucose through eliminating the glucose toxicity. There were shorter time to get blood glucose achieving standard by insulin treatment than OHA treatment. Moreover the insulin therapy can improve the β cell function by partly restoring the first phase of insulin secretion, and facilite to regulate insulin secretion mode of the beta cell. This suggests that the insulin treatment might be used as primary approach in the management of newly diagnosed type 2 diabetes patients.

0991

Diabetes treatment satisfaction as an independent factor contributing to glycaemic control in patients with type 2 diabetes mellitus

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Background and Aims: Health-related quality of life (HRQoL) and treatment satisfaction are typically reported directly by patients without physician interpretation and can be uniquely informative of the patient's perspective on effectiveness of therapy. We included patient-reported outcomes (PROs) in a recent clinical trial evaluating two dosing regimens of insulin glulisine (GLU) to assess whether PRO measures uniquely contribute to glycemic control in patients with type 2 diabetes mellitus (DM).
Materials and Methods: A multicenter, randomized, parallel-group, 24-wk study compared glycemic control in 273 patients with type 2 DM receiving basal insulin glargine (GLAR) and either 1) premeal GLU doses based on carbohydrate counting (Carb Count group; n=137) or 2) premeal GLU doses adjusted weekly to target according to a simple algorithm based on preprandial glucose patterns (ALG group; n=136). At baseline (BL), wk12, and wk24, patients were assessed on treatment satisfaction and HRQoL using the Diabetes Treatment Satisfaction Questionnaire (DTSQ), the Audit of Diabetes Dependent Quality of Life (ADDQoL) scale, and the Well-Being Questionnaire (W-BQ12).
Results: Patient characteristics at BL included a mean age of 55 y, 56% female, 81% white, 11% black, and mean A1c=8.16%. Only BL BMI was significantly different between groups, higher in the ALG group (37.73) than in the Carb Count group (35.56; p=0.019). Both treatment arms achieved target A1c at study end (ALG: 6.70%; Carb Count: 6.54%). The ALG group used higher daily doses of GLU (110.2 vs 94.3U; p=0.04), GLAR (103.4 vs 87.0U; p<0.0001), and total insulin (212.2 vs 181.1U; p=0.002). The ALG group had less symptomatic hypoglycemia<50 mg/dL than the Carb Count group (4.9 vs 8.0 events/pt-y; p=0.02). Patients' self-reported treatment satisfaction improved in both groups (p< 0.0001). Treatment satisfaction contributed independently to glycemic control; patients who were dissatisfied at study midpoint (wk12) were significantly more likely than satisfied patients to have higher average A1c values (p=0.0073). This contribution was above and beyond the effects of patient demographics, BL A1c, history of diet and carb counting, titration group, visits, and status of early study withdrawal. In addition, we found that patients in the ALG group who had a prior history of maintaining a healthy diet and practicing carb counting had the lowest mean A1c (6.46%, interaction with p=0.0174) over the course of the study.
Conclusions: In these patients with type 2 DM, treatment satisfaction with insulin therapy was independently related to glycemic control above and beyond other clinical variables such as baseline A1c and treatment arm. Greater diabetes treatment satisfaction was associated with improved A1c in patients receiving basal:bolus insulin therapy. Therefore, insulin therapy might be most efficacious for improving glycemic control when there is direct concern for patient satisfaction throughout treatment.
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0992

*H*yperglycaemia and its *e*ffect after *a*cute myocodial infraction on ca*r*diovascular (CV) outcomes in patients *t*ype *2 di*abetes mellitus (HEART2D): Baseline characteristics

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Background and Aims: Cardiovascular disease (CVD) is the major cause of morbidity and mortality in patients with type 2 diabetes mellitus (T2DM). Epidemiological studies indicate that the risk of CVD is associated with postprandial hyperglycemia, independently from fasting hyperglycemia, hemoglobin A1c (A1C), or other CV risk factors. The present study is designed to assess the effects of two different insulin regimens, one targeting postprandial (pp) blood glucose (BG) and the other targeting fasting/pre-meal BG, on CV outcomes up to 5 years after enrollment in patients with T2DM and acute myocardial infarction (AMI). Here we present characteristics of the study population at baseline.
Materials and Methods: 1120 patients from 105 study centres in 17 countries were randomised to 1 of 2 treatment strategies: (1) *prandial strategy*: premeal insulin lispro with basal insulin (NPH insulin) at bedtime if needed, targeting 2-h pp BG<7.5 mM or (2) *basal strategy*: NPH twice-daily or insulin glargine once-daily, followed by premixed human insulin twice-daily if needed, and targeting fasting and premeal BG (<6.7 mM). Both groups aim for a target AlC of <7%. The anticipated difference in pp BG (2.0 to 2.5 mM) between strategies is expected to demonstrate approximately 18.5% relative CV risk reduction in the prandial strategy group.
Results: At baseline patients' mean (±SD) for age was 61.0 (±9.8) years, body mass index was 29.1 (±4.8) kg/m^2, duration of diabetes was 9.1 (±7.2) years, 63% were male, and A1C was 8.3 (±1.5)%. Treatment for diabetes prior to AMI included: diet and exercise (9%), oral antihyperglycemic medications (sulfonylurea 26%, metformin +sulfonylurea 15%, biguanides 7%) and insulin (conven-

tional regimens 22%, MDI 6%). 15.7% patients had background retinopathy (proliferative retinopathy 4%), 21% had peripheral neuropathy, 9% had nephropathy, and 1.6% had a history of amputation. After entry AMI, reperfusion was immediately attempted in 58% of patients: primary percutaneous transluminal coronary angiopathy (PTCA) (43%), fibrinolysis (18%) and/or coronary artery bypass grafting CABG (3%). The mean left ventricular ejection fraction for the study population was 50.8 (±10)%.
Conclusion: When compared to the baseline demographic and clinical characteristics of patient populations from other interventional trials, HEART2D study population shows many typical features of patients with T2DM and AMI. Epidemiological evidence indicates that these individuals are at very high risk of recurrent CV events and death. Therefore, results of HEART2D may have important implications for the management of a broader population of people with T2DM complicated by coronary heart disease.
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PS 84

Insulin trials in type 1 or type 2 diabetes

0993

Transfer of patients with type 1 diabetes from NPH insulin to insulin glargine as basal insulin: a multicentre, randomised, parallel-group, open-label study

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Background and Aims: Once-daily (OD) basal insulin glargine can be used as part of a simple multiple daily injection (MDI) regimen in type 1 diabetes (T1DM) patients. This randomized, multicentre study compared the use of glargine and NPH insulin to normalize fasting plasma glucose (FPG; target 5.0–6.6 mmol/L [90–120 mg/dL]) during the last month (Month 6) of treatment.
Materials and Methods: Patients with T1DM were treated for 6 months with prandial insulin lispro plus either OD basal glargine (n=85) or twice-daily (BD) NPH (n=90) in MDI regimens (baseline characteristics given in Table). The primary outcome measures were FPG and mean amplitude of glycaemic excursion (MAGE).
Results: HbA_{1c} decreased from baseline to endpoint with glargine (mean±SD; 7.9±0.7 to 7.3±0.7%) and NPH (7.9±0.6 to 7.3±1.0%) (p=N/S between glargine and NPH). During the last month of treatment, decreases in mean daily FPG from baseline were significantly greater with glargine versus NPH (mean±coefficient of variation; 10.2±1.3 to 8.7±1.1 mmol/L [184.3±24.3 to 156.3±20.0 mg/dL] vs 10.4±1.3 to 9.9± 1.3 mmol/L [188.1±24.1 to 178.3±24.0 mg/dL]; $p<0.05$). Similarly, the decrease in MAGE was significantly greater with glargine versus NPH (mean±coefficient of variation; 8.2±1.5 to 7.1±1.9 mmol/L [148.3±26.6 to 128.1± 33.9 mg/dL] vs 7.9±1.9 to 7.7±2.0 mmol/L [143.0±34.2 to 139.5± 35.8 mg/dL]; $p<0.05$). In contrast, there was no significant difference with glargine versus NPH with respect to confirmed serious hypoglycaemic events (blood glucose<2.3 mmol/L [<41 mg/dL]) per patient-week over the last month of treatment (0.15 vs 0.17 events). The low incidence of severe hypoglycaemia in both groups prevented meaningful comparison.
Conclusion: In this study of patients with T1DM, where basal insulin was insufficiently titrated to target, OD glargine was still superior to BD NPH with lower FPG and lower glucose variability despite similar HbA_{1c}.

Baseline characteristics

	Insulin glargine	**(n=85)**	**NPH insulin**	**(n=90)**
	median	(min-max)	median	(min-max)
Male/female (%)	59/41		54/46	
Age (years)	35.5	18.0–57.0	36.3	19.0–58.0
BMI (kg/m^2)	23.4	18.9–27.9	23.5	18.9–27.6
Duration of diabetes (years)	13.1	1.2–37.6	14.3	2.4–48.1
FBG (mmol/L)	10.2	5.0–16.8	10.3	5.4–16.8
[mg/dL]	[184]	[91–303]	[186]	[97–302]
HbA_{1c} (%)	7.8	5.9–9.8	7.8	6.4–9.0

Data are medians with minimum-maximum values except male/female ratio (%)

Supported by sanofi-aventis

0994

Time of evening administration of insulin detemir can be fitted to individual needs and lifestyle

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Background and Aims: Studies of blood glucose control with insulin detemir (detemir) vs. NPH insulin (NPH) with insulin aspart at meal-times have been subjected to meta-analyses.

Materials and Methods: Data were included from two 16-week randomised, multi-national, parallel, open-label trials in 808 people with Type 1 diabetes (mean age 40 yr, HbA_{1c} 8.3%, BMI: 25.2 kg/m^2 and diabetes duration 16 yr). Both NPH and detemir were given morning+bedtime (NPH, $detemir_{bed}$) but additionally detemir was given morning+evening (morning+dinner or at 12-h intervals), ($detemir_{eve}$).

Results: Baseline-adjusted HbA_{1c} was significantly lower with $detemir_{eve}$ (7.71%) than with NPH (7.85%) (Table 1) at 16 weeks, and possibly lower with $detemir_{bed}$ (7.72%). The two detemir arms were comparable. Clinic fasting plasma glucose (FPG) was lower with $detemir_{bed}$ than with $detemir_{eve}$ (9.0 vs. 9.8 mmol/L), and both were lower compared to NPH (11.2 mmol/L). Within-participant variation in self-measured FPG was significantly lower with detemir (both arms) than with NPH (SD 2.7 vs. 3.3 mmol/L, $p<0.001$). Self-measured plasma glucose during the night was higher with $detemir_{bed}$ than with $detemir_{eve}$ and the risk of nocturnal hypoglycaemia was 29 and 26% lower with $detemir_{bed}$ than with $detemir_{eve}$ and NPH respectively.

Table 1: Estimated treatment differences

HbA_{1c} (%)	Difference	95% CI	p
$detemir_{eve}$ - NPH	−0.13	[−0.26; −0.00]	0.043
$detemir_{bed}$ - NPH	−0.13	[−0.25; 0.00]	0.053
$detemir_{eve}$ - $detemir_{bed}$	−0.00	[−0.13; 0.12]	NS
FPG (mmol/L)			
$detemir_{eve}$ - NPH	−1.35	[−1.83; −0.87]	<0.001
$detemir_{bed}$ - NPH	−2.14	[−2.62; −1.65]	<0.001
$detemir_{eve}$ - $detemir_{bed}$	0.78	[0.31; 1.26]	0.001
Night-time hypoglycaemia (Relative Risk)			
NPH/$detemir_{eve}$	0.96	[0.74; 1.25]	NS
$detemir_{bed}$/NPH	0.74	[0.57; 0.96]	0.026
$detemir_{bed}$/$detemir_{eve}$	0.71	[0.55; 0.92]	0.011

Conclusion: The time of evening administration of insulin detemir can be varied to match the needs and wishes of the individual patient.

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0995

Insulin detemir achieves lower, less variable fasting glucose and a reduced risk of nocturnal hypoglycaemia and weight gain compared to NPH insulin in basal bolus therapy of Japanese patients with type 1 diabetes

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Background and Aims: Insulin detemir has been associated with reductions in fasting plasma glucose (FPG) variability, risk of hypoglycaemia and weight gain in comparison with NPH insulin in a series of phase 3 studies. The present investigation sought to assess the clinical profile of insulin detemir in a Japanese cohort, and to test for non-inferiority in comparison with NPH insulin.

Materials and Methods: This 48-week, multi-centre, open-label, parallel-group trial asymmetrically randomised and exposed 396 patients with type 1 or type 2 diabetes (body mass index [BMI] <30 kg/m^2) previously established on basal-bolus therapy, to receive mealtime insulin aspart with either insulin detemir or NPH insulin, each given once or twice daily, with continuous titration. The majority of patients had type 1 diabetes (insulin detemir, n=196; NPH insulin, n=98), hence the outcomes of this subgroup were assessed independently, and the key data are presented here. Key demographic characteristics of this group included: mean age, 42.2 years; mean duration of diabetes, 13.3 years; mean HbA_{1c}, 7.41%; mean BMI 22.4 kg/m^2. Demographic characteristics were well matched by treatment.

Results: HbA_{1c} at endpoint did not differ between treatments, the baseline-adjusted means at endpoint being 7.33% and 7.29% for insulin detemir and NPH insulin, respectively (point estimate [95% CI] for detemir - NPH=0.03% [−0.14; 0.21], confirming non-inferiority). There were small respective decreases in HbA_{1c} of −0.08% and −0.12% from baseline values. FPG at endpoint was lower with insulin detemir than NPH insulin, with baseline-adjusted means at endpoint being 146.5 mg/dl and 157.0 mg/dl (p=0.0263), respectively (8.14 mmol/l and 8.72 mmol/l). There were respective decreases in FPG of

−24.4 mg/dl and −17.4 mg/dl (1.36 mmol/l and 0.97 mmol/l). Within-subject variability (SD) of FPG was also lower at endpoint with insulin detemir (53.4 mg/dl vs. 63.6 mg/dl, $p<0.0001$; 2.97 mmol/l vs. 3.53 mmol/l).The overall incidence of hypoglycaemia was ~51 episodes/patient-year in both groups, but the risk for nocturnal hypoglycaemia was lower with insulin detemir (Relative Risk, 0.69, $p=0.0464$). Baseline-adjusted weight was lower in insulin detemir-treated patients at endpoint (point estimate for detemir-NPH: −0.92 kg, $p=0.0024$). There were no between-group differences in other safety parameters. The mean daily basal insulin dose at end of trial was significantly lower with insulin detemir ($p=0.0001$), the ratio (insulin detemir/NPH insulin) being 0.869. However, the dose of bolus insulin was slightly higher in the insulin detemir group (NS), hence the ratio for total daily insulin dose at end of trial was 0.983.

Conclusion: Insulin detemir, as the basal component of basal-bolus therapy, was associated with several clinical advantages in comparison with NPH insulin in Japanese patients with type 1 diabetes. These advantages included better fasting glucose control, with lower variability, fewer nocturnal hypoglycaemic episodes and reduced weight gain.

Supported by Novo Nordisk Pharma Ltd

0996

Once-daily insulin detemir added to oral antidiabetic drugs results in less weight gain and a trend for reduced hypoglycaemia in comparison to NPH insulin in Japanese patients with type 2 diabetes

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Background and Aims: Patients with type 2 diabetes usually benefit from addition of insulin therapy when hyperglycaemia is no longer adequately controlled by oral antidiabetic drugs (OADs). A popular, simple regimen for initiating insulin is to add a once daily injection of a long acting insulin preparation to existing therapy, titrating dose towards guideline targets. This study compared efficacy and tolerability of once-daily insulin detemir with NPH insulin used in this way in Japanese patients, seeking to confirm non-inferiority of insulin detemir.

Materials and Methods: This 36 week, multi-centre, open-label, parallel group trial randomised and exposed insulin naïve adults with type 2 diabetes diagnosed ≥1 year, with BMI<30 kg/m^2, and HbA_{1c} ≥7.5%,<10.0%, to receive a once-daily bedtime injection of insulin detemir (n=180) or NPH insulin (n=183), in addition to their existing 1–3 OADS. All patients were on sulphonylureas,+/− biguanides and/or alpha glucosidase inhibitors, with all doses within approved Japanese labelling. Insulin was titrated against pre-breakfast plasma glucose (FPG), the dose increased in small steps if this remained ≥120 mg/dl and decreased if it was below 70 mg/dl. The goal was to aim for Japanese Diabetes Association guidelines for 2002–2003 for good or excellent glycaemic control (HbA_{1c} ≤6.4%, FPG ≤119 mg/dl), without hypoglycaemia.

Results: Glycaemic control was similar for both basal insulins at end of trial. Adjusted endpoint mean (SE) values for HbA_{1c} were 7.81% (0.07) and 7.74% (0.07) for insulin detemir and NPH insulin, respectively (point estimate [95% CI], detemir-NPH,=0.07% [−0.07; 0.21]), confirming non-inferiority of insulin detemir. There were respective mean decreases in HbA_{1c} of −0.59% and −0.67%. The adjusted endpoint mean (SE) values for FPG were 125.38 mg/dl (2.97) and 123.32 mg/dl (2.90) for insulin detemir and NPH insulin (6.97 mmol/l and 6.85 mmol/l), respectively (point estimate [95% CI], detemir-NPH,=2.06 mg/dl [−3.95; 8.07]). There were respective mean decreases in FPG of −41.88 mg/dl and −43.48 mg/dl (2.33 mmol/l and 2.42 mmol/l). There was a trend for a reduced incidence of hypoglycaemia in recipients of insulin detemir, compared with NPH insulin: 4.51 vs. 6.46 events/subject/year, relative risk 0.71 ($p=0.0636$). Similarly, respective incidences for nocturnal hypoglycaemia were 1.12 vs. 2.10 events/subject/year, relative risk 0.60 ($p=0.0754$). Weight gain was lower with insulin detemir (0.78 kg) than NPH insulin (1.18 kg), adjusted endpoint means (SE): 61.26 kg (0.18) and 61.64 (0.18) respectively ($p=0.0392$). No between-group differences in other safety parameters were apparent. Mean daily insulin dose was slightly, but statistically significantly, higher in recipients of insulin detemir than NPH insulin (ratio, 1.19).

Conclusion: Once-daily insulin detemir added to OAD therapy in Japanese patients with poorly controlled type 2 diabetes improves glycaemic control to the same extent as NPH insulin, but with reduced weight gain and a tendency for fewer hypoglycaemic episodes.

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0997

The PREFER Study: a comparison of outcomes in patients using insulin detemir once daily or twice daily in a basal bolus regimen with mealtime insulin aspart

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Background and Aims: IDet is a long-acting basal insulin analogue often used in basal-bolus regimens. At present it is unclear what proportion of patients need IDet twice daily (BID), rather than once daily (OD), and whether or not they achieve the same clinical outcomes. In this study designed to compare a basal-bolus regimen with a BID regimen of premixed analogue BIAsp 30, dosing and outcome data in the basal-bolus arm were analysed for patients that received IDet OD or BID in addition to mealtime IAsp.
Methods and Materials: Poorly controlled ($7 \leq HbA_{1c} \leq 12\%$) patients with type 2 diabetes previously treated with OADs alone, or in combination with basal insulin (glargine or NPH), were included in this 26-week, multi-centre, open-label, parallel-group trial. In the IDet/IAsp group (n=537), IDet was initiated once daily plus IAsp three times daily (distributed 3:1:2 breakfast:lunch:dinner). In patients pre-treated with basal insulin, the previous dose was switched 1:1 unit-for-unit for IDet, while in insulin-naïve patients, initial IDet dose was 10–14 U. OADs were discontinued. Insulin doses were titrated to predefined plasma glucose (PG) targets using an algorithm. IDet was titrated to ≤7.0 mmol/l prebreakfast and IAsp to 90 min postprandial PG ≤10 mmol/l. If predinner PG exceeded 7.0 mmol/l on >5 consecutive days and/or nocturnal hypoglycaemia prevented adequate titration of the dinner IDet dose, a second IDet dose was added prebreakfast and titrated to predinner PG ≤7.0 mmol/l.
Results: In the IDet/IAsp group, 87% of patients remained on once-daily IDet throughout the trial. End-of-trial IDet doses were 0.353 U/kg when used once daily, and 0.557 U/kg when used twice daily (0.201 U/kg at breakfast, 0.356 U/kg at dinner). End-of-trial (EOT) *total* daily insulin dose was 0.86 U/kg (0.38 U/kg IDet, 0.48 U/kg IAsp). For IAsp, mean initial dose was 20.2 U and final dose split was: breakfast 35% (0.173 U/kg), lunch 29% (0.140 U/kg) and dinner 36% (0.176 U/kg). Glycaemic control (HbA_{1c}) was similar for patients receiving IDet OD or BID: 6.93% and 6.97%, respectively; p=0.697. Fasting plasma glucose (FPG) measured during the last week of the trial was 7.46 mmol/l for OD IDet and 8.00 mmol/l for BID IDet. The respective FPG coefficients of variation were 12.91% and 15.34%. At EOT there was no significant difference in body weight between OD and BID IDet-treated patients: 91.7 vs. 92.2 kg, respectively; p=0.305. EOT BMI was also similar: 31.8 (OD IDet) vs. 32.0 (BID IDet); p=0.316. The proportion of patients that experienced any hypoglycaemia was 48.5% for OD and 42.2% for BID IDet. Minor nocturnal hypoglycaemia was similar for OD vs. BID IDet: 7.3% vs. 6.3% of patients.
Conclusions: OD dosing of IDet was effective for the majority of patients in a treat-to-target basal-bolus regimen with IAsp at mealtimes. Patients using IDet OD or BID achieved similar glycaemic control, body weight and BMI, and experienced similar rates of hypoglycaemia.
Supported by Novo Nordisk

0998

The PREFER study: both biphasic insulin aspart 30 twice-daily and basal-bolus using insulin detemir and insulin aspart enabled patients with type 2 diabetes to achieve HbA_{1c} target<7.0%

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Background and Aims: Insulin analogues are widely used in type 2 diabetes management but rarely compared in clinical trials. This study evaluated two insulin analogue regimens in patients with type 2 diabetes: biphasic insulin aspart 30 (BIAsp 30, 30% insulin aspart, 70% protaminated aspart) and basal-bolus (BB) therapy using insulin detemir (IDet) and insulin aspart (IAsp).
Materials and Methods: This was a 26-week, treat-to-target, multicentre, randomised, open-label, parallel-group trial of 715 subjects ($7 \leq HbA_{1c} \leq 12\%$) previously treated with OADs alone (72%) or in combination with glargine or NPH insulin. BIAsp 30 (n=178) was initiated twice daily (0.2U/kg at breakfast, 0.1U/kg at dinner). BB therapy (n=537) was initiated with IDet once daily (10U or 14U if BMI >32 kg/m^2) plus IAsp with meals (dosed individually in ratio 3:1:2 breakfast:lunch:dinner). OADs were discontinued in both arms. BIAsp 30 was titrated to plasma glucose (PG) target ≤7.0 mmol/l prebreakfast and predinner. IDet (administered predinner) was titrated to PG ≤7.0 mmol/l prebreakfast and IAsp to 90 min postprandial PG ≤10 mmol/l. If pre-dinner PG was elevated for 5

consecutive days and/or nocturnal hypoglycaemia prevented adequate titration of the IDet dose, a second IDet dose was added prebreakfast and titrated to PG ≤7.0 mmol/l predinner.

Results: In the IDet/IAsp group, initial mean dose for IAsp was 20.2 U. At end of trial, mean total daily doses were 0.63U/kg for BIAsp 30 and 0.86U/kg for IDet/IAsp. Of the IDet/IAsp patients, 87% remained on once-daily IDet. Reduction in HbA_{1c} was significantly greater in patients on IDet/IAsp (8.52% to 6.96%) than on BIAsp30 (8.40% to 7.17%); baseline-adjusted HbA_{1c} difference (IDet/IAsp-BIAsp 30) 0.23% (p=0.0052). More than half of all patients reached HbA_{1c} ≤7.0%: 50% of the BIAsp 30 group and 60% of the IDet/IAsp group. Patients previously treated with insulin had greater HbA_{1c} reduction with IDet/IAsp than with BIAsp 30 (1.21% vs 0.75%; p=0.0129), while insulin naive patients had similar HbA_{1c} reductions (1.69% vs 1.42%; p=0.106). Mean fasting PG (FPG) was similar between groups: 8.05 vs 8.27 mmol/l (BIAsp 30 vs. IDet/IAsp; p=0.345). Intrasubject coefficients of variation for FPG were low for both regimens: BIAsp 30, 12.6%; IDet/IAsp, 13.3%. No patients on BIAsp 30 experienced major hypoglycaemia, compared with 5 patients (0.9%) on IDet/IAsp. Minor hypoglycaemia (PG<3.1 mmol/l) occurred in 28% of BIAsp 30 and 31% of IDet/IAsp patients. Nocturnal minor hypoglycaemia occurred in 7.3% and 7.4% of patients, respectively. Both groups showed a similar increase in body weight from baseline (BIAsp 30: 2.1 kg; IDet/IAsp: 2.4 kg).

Conclusions: Overall, in poorly-controlled patients with type 2 diabetes, premixed and BB insulin analogue regimens enabled 58% to reach HbA_{1c} ≤7%. BB treatment with IDet/IAsp resulted in a significantly greater HbA_{1c} reduction than twice-daily BIAsp 30 in previously insulin-treated patients, but insulin-naïve patients achieved equal glycaemic control on each regimen. Hypoglycaemia rates were low and similar between treatments.

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0999

The PREFER Study: Dosing recommendations for biphasic insulin aspart 30, insulin detemir and insulin aspart using treat-to-target titration

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Background and Aims: Premixed and basal-bolus insulin analogue regimens are widely prescribed for the treatment of type 2 diabetes but few data are available on dose distribution. In this treat-to-target study, dosing data were analysed from 715 patients receiving premixed analogue, biphasic insulin aspart 30/70 (BIAsp 30; 30% insulin aspart, 70% protaminated aspart), or all-analogue basal-bolus therapy using insulin detemir and insulin aspart (IDet/IAsp).

Materials and Methods: Prior to randomisation in this 26-week, multi-centre, open-labelled, parallel-group trial, poorly controlled (7≤HbA_{1c}≤12%) patients with type 2 diabetes were treated with OADs alone or in combination with basal insulin (insulin glargine or NPH insulin). In the BIAsp 30 group (n=178), insulin was initiated twice daily at 0.3 U/kg: 2/3 of dose at breakfast, 1/3 at dinner, irrespective of pre-trial treatment. In the basal-bolus group (n=537), IDet was initiated once daily plus IAsp three times daily with meals (in ratio 3:1:2 breakfast:lunch:dinner). In patients previously treated with basal insulin, the IDet dose was a 1:1 unit-for-unit switch, while in insulin-naïve patients, initial IDet dose was 10 U or 14 U if BMI >32 kg/m^2. All OADs were discontinued in both groups. Insulin dose adjustment was algorithm-driven to predefined plasma glucose (PG) targets. BIAsp 30 was titrated to ≤7.0 mmol/l prebreakfast and predinner. IDet was titrated to ≤7.0 mmol/l pre-breakfast and IAsp to 90 min postprandial PG ≤10 mmol/l. If pre-dinner PG exceeded 7.0 mmol/l on > 5 consecutive days and/or nocturnal hypoglycaemia prevented adequate titration of the dinner IDet dose, a second IDet dose was added pre-breakfast and titrated to pre-dinner PG ≤7.0 mmol/l.

Results: Mean total daily insulin doses after 26 weeks were 0.63U/kg for BIAsp30 and 0.86 U/kg for IDet/IAsp (0.38 U/kg IDet, 0.48 U/kg IAsp; initial mean IAsp dose was 20.2 U). Titration of BIAsp 30 resulted in a 50:50 dose split between breakfast and dinner: 0.315 and 0.316 U/kg, respectively. For IAsp, the final dose split between meals was: breakfast 35% (0.17 U/kg), lunch 29% (0.14 U/kg) and dinner 36% (0.18 U/kg). In the IDet/IAsp group, 87% of patients remained on once-daily IDet throughout the trial. End-of-trial IDet doses were 0.35 U/kg when used once daily, and 0.56 U/kg when used twice daily (0.20 U/kg at breakfast, 0.36 U/kg at dinner). After 26 weeks, HbA_{1c} was 6.96% for the IDet/IAsp group and 7.17% for BIAsp 30, baseline-adjusted HbA_{1c} difference (IDet/IAsp-BIAsp 30): 0.23%; p=0.0052. Glycaemic control (HbA_{1c}) was similar for patients receiving IDet once or twice daily: 6.97% and 6.93%, respectively; p=0.697.

Conclusions: Titration of twice-daily BIAsp 30 and three-times-daily IAsp (in an analogue basal-bolus regimen) resulted in equal distribution of doses between meals. Only 13% of IDet/IAsp patients required twice-

daily IDet, showing once-daily dosing was effective for most patients.
Supported by Novo Nordisk A/S

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Pharmacokinetics and pharmacodynamics of biphasic insulin aspart 50 and biphasic insulin aspart 70 in healthy Japanese subjects

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Background and Aims: Biphasic insulin aspart (BIAsp) is a premixed insulin analogue formulation comprising a proportion of soluble insulin aspart (IAsp) in association with protamine-retarded IAsp. The soluble IAsp fraction is rapidly absorbed after subcutaneous injection, providing prandial glucose control, while the delayed absorption of the protamine-retarded fraction provides basal insulin coverage. In clinical trials, BIAsp 30 (30% soluble IAsp; 70% protamine-retarded IAsp) provides effective glycaemic control when administered twice-daily before meals. The rationale for developing BIAsp 50 and BIAsp 70 (50% and 70% soluble IAsp; 50% and 30% protamine-retarded IAsp, respectively) is to provide the possibility of premixed insulin analogues for administration twice or three times daily in patients with additional need for controlling post-prandial glucose or varying diets and lifestyles. The present investigation compared the pharmacokinetics and pharmacodynamics of BIAsp 50 and BIAsp 70.
Materials and Methods: A randomised, open-labelled, two-period crossover trial involving 24 healthy Japanese male subjects (age: 20–50 years; body mass index: 19–27 kg/m^2; fasting blood glucose: 3.8–6.0 mmol/L). Subjects received a single dose (0.8 U/kg) of BIAsp 50 or BIAsp 70 at the start of the first treatment period. Subjects then received the same dose of the alternate trial drug at the start of the second treatment period, scheduled 6–12 days after the first. A 28-point (24-hour) serum insulin aspart (IAsp) profile and a 21-point (10-hour) serum glucose profile were determined after dosing. Endpoint analysis was performed using ANOVA.
Results: Maximum insulin concentration ($C_{max\ (ins,\ 0–24\ h)}$) was higher for BIAsp 70 than for BIAsp 50 (214.1 vs 153.7 pmol/L; p<0.0001). Correspondingly, the maximum glucose-lowering effect for BIAsp 70 was significantly greater than for BIAsp 50 (delta $C_{min\ (glu,\ 0–10\ h)}$, 1.664 vs. 1.250 mmol/L; p<0.0001). Minimum glucose levels were achieved 45 minutes earlier with BIAsp 70 than with BIAsp 50 (p<0.05). Furthermore, mean residence time was statistically shorter for BIAsp 70 than for BIAsp 50 (1.986 vs. 3.683 h; p=0.0001). Overall exposure to IAsp was similar between treatments ($AUC_{ins,\ 0-\infty,}$ BIAsp 50, 442.0; BIAsp70, 435.7 pmol/h/L) although exposure to IAsp over the first 4 hours after dosing ($AUC_{ins,\ 0–4\ h}$) was greater for BIAsp 70 than for BIAsp 50 (379.0 vs. 304.2 pmol/h/L; p<0.0001). The overall glucose-lowering effect was similar between treatment groups ($AOC_{(glu,\ 0–10\ h)}$, BIAsp 70, 6.747; BIAsp 50, 6.514 mmol/h/L; n.s.).
Conclusion: Maximum IAsp levels were higher for BIAsp 70. This could be predicted due to the greater proportion of soluble IAsp in BIAsp 70. Accordingly, the increased proportion of soluble IAsp in BIAsp 70 than in BIAsp 50 resulted in a significantly greater initial glucose-lowering effect with BIAsp 70. However, overall exposure to IAsp and the overall glucose-lowering effect was similar between treatments. In summary, the pharmacokinetic and pharmacodynamic properties of BIAsp 50 and BIAsp 70 reflected the proportions of soluble and protamine-retarded IAsp in the formulations. In clinical practice, the enhanced initial glucose-lowering effect of BIAsp 70 may offer improved control of postprandial blood glucose levels as compared with BIAsp 50.
Supported by Novo Nordisk Pharma Ltd

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Early study termination is related to patient-reported outcomes during therapy with mealtime insulin glulisine in patients with type 2 diabetes mellitus

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Background and Aims: Participants may terminate early from a clinical trial for reasons such as “do not wish to continue,” “lost to follow-up,” and “study-related or -unrelated adverse events.” While study-specific factors related to early termination may impact study completion rates and trial success, the effect of more stable factors such as measures of clinical and patient-reported outcomes (PROs) may have implications beyond the trial for real-life treatment success.
Materials and Methods: A multicenter, randomized, parallel-group, 24-wk study compared glycemic control in 273 patients with type 2 diabetes mellitus (DM) who, in

addition to receiving basal insulin glargine (GLAR) throughout, received either 1) premeal insulin glulisine (GLU) at doses based on carbohydrate counting (Carb Count group; n=137) or 2) premeal GLU at doses adjusted weekly to target according to a simple algorithm based on preprandial glucose patterns (ALG group; n=136). At baseline (BL), wk12, and wk24, patients were assessed on treatment satisfaction and health-related quality of life (HRQoL), using the Diabetes Treatment Satisfaction Questionnaire (DTSQ), the Audit of Diabetes Dependent Quality of Life (ADDQoL) scale, and the Well-Being Questionnaire (W-BQ12).

Results: Patient characteristics at BL included a mean age of 55 y, 56% female, 81% white, 11% black, and mean A1c=8.16%. Only BL BMI was significantly different between groups, higher in the ALG group (37.73) than in the Carb Count group (35.56; p=0.019). Both treatment arms achieved target A1c at study end (ALG: 6.70%; Carb Count: 6.54%). The ALG group had less symptomatic hypoglycemia<50 mg/dL than the Carb Count group (4.9 vs 8.0 events/pt-y; p=0.02). Twelve ALG patients and 28 Carb Count patients withdrew from the study before the last visit. Patients who stayed in the study reported greater treatment satisfaction than patients who withdrew (p=0.0002). Specifically, early termination was more likely among patients who were randomized to Carb Count (p=0.0361), younger (p=0.0003), had higher BL A1c (p=0.0012), or no history of a healthy diet prior to the study (p<0.0001). Furthermore, the relationships between HRQoL and treatment satisfaction and early study termination were largely dependent on the timing of assessment. Patients reporting greater negative impact of diabetes on HRQoL at BL were less inclined to withdraw from the study (p=0.002). However, if such impact was reported at wk12, patients were more likely to withdraw (p=0.0015). Patients reporting dissatisfaction at wk12 due to hyperglycemic events were more inclined to withdraw if in the Carb Count group (p=0.0027). Treatment satisfaction at wk12 was unrelated to early termination in the ALG group.

Conclusions: Early study termination is correlated with baseline A1c and patient-reported outcomes, beyond study-specific factors. We found that patient reports of poor HRQoL at baseline are associated with a higher chance of study completion, while poor HRQoL at mid-study increases the chance of early termination. It appears that patients may have attributed HRQoL to past or present treatment success, which is, in turn, related to early study termination.

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Safe and effective control with insulin in patients with type 2 diabetes mellitus using two different methods of titrating mealtime glulisine

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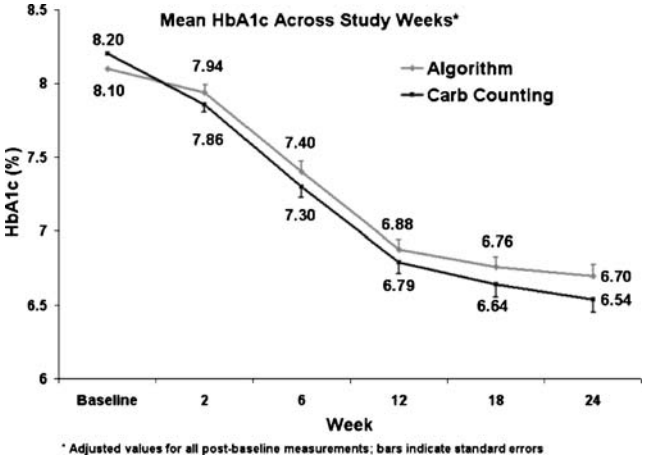
Background and Aims: This open-label, multicenter, randomized, 24-wk study tested two methods of titrating mealtime insulin glulisine (GLU): (ALG; n=136) titration based on preprandial blood glucose (BG) patterns (number of BG values above or below the premeal target) with dosing adjustments based on a simple algorithm method (adding 1, 2, or 3 units (U) depending on specific mealtime dose) vs (Carb Count; n=137) titration based on preprandial BG patterns and insulin to carbohydrate (I:C) ratio changes with dosing adjustments based on the carbohydrate content of a meal.

Materials and Methods: Patients had type 2 diabetes mellitus (DM) uncontrolled on 2 or more insulin injections (age 55.1 y; duration 13 y; BMI 36.7 kg/m^2; A1c 8.2%). Patients were switched to basal:bolus therapy with daily glargine (GLAR), titrated to fasting BG<95 mg/dL, and GLU before meals,±metformin, with targets of<100 mg/dL prelunch/dinner and<130 mg/dL at bedtime. Premeal GLU was adjusted weekly. ALG added 1, 2, or 3U based on premeal glucose patterns; Carb Count adjusted the I:C ratio.

Results: At wk24, A1c was significantly reduced in both groups (p<0.0001); ALG and Carb Count did not differ in change from baseline A1c (−1.46% vs −1.62%; p=0.15), proportion of patients achieving A1c<7.0% (73.0% vs 69.2%; p=0.7), and weight gain (3.7 vs 2.4 kg; p=0.06). The ALG group used higher doses of GLU (110.2 vs 94.3U; p=0.04) and GLAR (103.4 vs 87.0U; p<0.0001), and had less symptomatic hypoglycemia<50 mg/dL (4.9 vs 8.0 events/pt-y; p=0.02) than the Carb Count group. Both groups ended with a basal:bolus ratio of close to 50:50 and used 1.8–2 U/kg of insulin/day. Adverse events in each group were similar.

Conclusions: Both titration methods used for adjustments of mealtime GLU based on preprandial glucose patterns are safe and effective. GLU adjustment based on a simple algorithm method provides a new simplified alternative to the carb counting method.



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PS

85 Inhaled insulin

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Body weight changes associated with insulin therapy: A retrospective pooled analysis of inhaled human insulin (Exubera®) versus subcutaneous insulin in 5 controlled phase 3 trials

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Background and Aims: Weight gain is a frequent effect of insulin therapy and recent evidence suggests that fear of weight gain is associated with reduced adherence to diabetes medication. The aim of this study was to compare the effect of inhaled human insulin (INH, Exubera® (insulin human [rDNA origin]) Inhalation Powder) administered in combination with intermediate or long-acting subcutaneous (SC) insulin versus SC insulin regimens on body weight after 6 months of treatment in adult patients with type 1 (T1DM) or type 2 (T2DM) diabetes mellitus.

Materials and Methods: Retrospective analysis of pooled 6-month data from 5 controlled Phase 3 clinical trials conducted in the Americas.

Results: Less weight gain was observed with INH therapy compared to SC insulin in adult patients with T1DM or T2DM while changes in glycosylated hemoglobin (HbA_{1c}) were comparable in both treatment groups (Table). Hypoglycemia (T1DM [n=1048]: INH 6.981 vs SC 6.823 events/subject-month; T2DM [n=912]: INH 1.812 vs SC 2.077 events/subject-month) and severe hypoglycemia (T1DM: INH 5.829 vs SC 5.225 events/100 subject-months; T2DM: INH 0.781 vs SC 1.051 events/100 subject-months) were also comparable.

Conclusion: Less weight gain was observed with INH therapy compared to SC insulin in adult patients with T1DM or T2DM. It is possible that the pharmacokinetic profile of INH more closely approximates near physiologic prandial insulin replacement resulting in reduced hyperinsulinemia and, thus, less weight gain in the setting of comparable HbA_{1c} and incidence of hypoglycemia. Reduction in the fear of weight gain may be one factor that helps to encourage the adoption of insulin, especially in adult patients with T2DM who are frequently reluctant to accept insulin therapy despite the failure of oral agent therapy.

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6-Month Data	T1DM			T2DM		
	INH	SC	Treatment group difference (95% CI)	INH	SC	Treatment group difference (95% CI)
Mean±SD (n) body weight (kg) at baseline	76.0±14.0 (496)	75.0±13.4(510)	-	88.5±14.9 (423)	88.5±15.0 (428)	-
Mean (SE) adjusted change from baseline in body weight (kg)†	0.2 (0.1)	1.1 (0.1)	−0.87 (−1.24, −0.50)	0.7 (0.2)	1.6 (0.2)	−0.93 (−1.39, −0.48)
Mean±SD (n) HbA_{1c} (%) at baseline	7.6±1.0 (500)	7.7±1.0 (509)	-	7.8±1.1 (425)	7.9±1.1 (437)	-
Mean (SE) adjusted change from baseline HbA_{1c} (%)†	−0.2 (0.03)	−0.3 (0.03)	0.09 (0.00, 0.18)	−0.7 (0.05)	−0.6 (0.04)	−0.07 (−0.19, 0.05)

†least squares means based on the primary model with terms for baseline, treatment, and center.

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Sustained efficacy and tolerability of inhaled human insulin (Exubera®). Therapy over 2 years: patients with type 2 diabetes

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Background and Aims: The availability of inhaled human insulin (INH; Exubera® (insulin human [rDNA origin]) Inhalation Powder) may increase patient acceptance of insulin, improve glycemic control, and potentially reduce the burden of insulin therapy in diabetes. In patients with type 2 diabetes mellitus (T2DM), one previous 24-week study showed comparable efficacy between INH and subcutaneous (SC) insulin, and another 104-week study demonstrated superior glycemic control of INH compared with oral agents (OAs). Small treatment group differences in lung function (measured using routine pulmonary function testing [PFT]) were observed in both studies, but long-term studies are critical to better characterize pulmonary safety.The aim of this study was to investigate the long-term (2-year) efficacy and safety profile of INH therapy in patients with T2DM.
Materials and Methods: An open-label, parallel-group, multicenter study where patients (aged between 35 and 75 years) were randomized to INH (n=319) or SC insulin (n=316). Primary end point was pulmonary function measured using highly standardized PFT. Secondary end points included glycosylated hemoglobin (HbA_{1c}), hypoglycemia, fasting plasma glucose (FPG), and body weight.
Results: Treatment group differences in changes from baseline in forced expiratory volume in 1 second (FEV_1) and carbon monoxide diffusing capacity (DL_{co}) were small (<1.5% of mean baseline), occurred early (Month 3 data: −0.043 L [90% confidence interval (CI), −0.065, −0.020] for FEV_1 and −0.194 mL/min/mm Hg [−0.437, 0.050] for DL_{co}), and remained stable with no progression for up to 2 years (−0.023 [−0.047, 0.002] and 0.165 [−0.102, 0.432], for FEV_1 and DL_{co}, respectively). Glycemic control was maintained in both groups with HbA_{1c} improvements from 7.7% and 7.8% to 7.3% and 7.3% in INH and SC, respectively, and the percentage of patients who achieved target HbA_{1c} <7% was comparable between groups (INH, 47.5% and SC, 45.2%). The incidence of hypoglycemic events was lower with INH than with SC insulin (0.8 vs 1.0 events/subject-month, respectively), and the incidence of severe hypoglycemic events was comparable in the 2 groups (0.4 vs 0.6 events/100 subject-month, respectively). After 24 months, INH resulted in greater reductions in FPG compared with SC insulin (from 8.4 to 7.5 vs 8.2 to 8.2 mmol/L [151.2 to 135.6 vs 148.2 to 147.1 mg/dL], respectively), and less body weight gain (mean±SD, 1.7±4.7 kg vs 3.0±5.2 kg). Adverse events were generally comparable with the exception of cough in the INH group, which was mild in most patients, nonproductive, and closely associated with dosing.
Conclusion: This study demonstrated that INH is well tolerated, provides comparable glycemic control, less weight gain, and greater FPG reductions when compared with SC insulin over 2 years in adult patients with type 2 diabetes.
This study was funded by Pfizer Inc

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Single-dose pharmacokinetics of inhaled human insulin (Exubera®) after acute passive cigarette smoke exposure

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Background and Aims: The bioavailability of inhaled human insulin (INH; Exubera® (insulin human [rDNA origin]) Inhalation Powder) is increased in chronic smokers compared with nonsmokers. As INH could be used in circumstances where patients are passively exposed to cigarette smoke, this study investigated the effect of a single acute passive cigarette smoke exposure (PSE) on INH bioavailability.
Materials and Methods: In this open-label, randomized, crossover study, healthy, nonsmoking subjects (n=28) received a single INH dose (3 mg) with no PSE, or after 2 hours of PSE. To simulate PSE, a commercial smoking machine burning full flavor cigarettes generated environmental nicotine levels typical of social settings (75–125 μg/m^3). There was a ≥ 2 week washout between study phases. Blood samples were collected at specified times up to 6 hours after INH administration to assess insulin pharmacokinetics.
Results: The mean plasma insulin area under the curve ($AUC_{0–360}$) decreased from 5703 μU·min/mL with INH alone to 4718 μU·min/mL after PSE exposure; ratio PSE+INH/INH alone 82.7 (95% confidence interval [CI], 68.8, 99.5). The mean maximum concentration (C_{max}) also decreased after PSE: 41.0 μU/mL with INH alone to 28.9 μU/mL; ratio PSE+INH/INH alone 70.5 (95% CI, 59.8, 83.1). The median time to maximum concentration

(T_{max}) occurred 15 minutes later (75 vs 60 min), after PSE relative to no PSE. INH was generally well tolerated by volunteers in this study.

Conclusion: In contrast to the effects of active smoking, investigations using technetium labeled diethylenetriaminepentaacetate (DTPA) and findings from this study suggest that acute PSE causes a decrease in lung permeability. This decrease in INH bioavailability after acute PSE does not create a risk of hypoglycemia.

This study was funded by Pfizer Inc

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Absorption of inhaled human insulin (Exubera®) after 3 and 13 weeks of active smoking cessation

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Background and Aims: Previous studies have demonstrated that bioavailability of inhaled human insulin (INH; Exubera® (insulin human [rDNA origin]) Inhalation Powder) is greater in chronic smokers relative to nonsmokers. The effects of smoking cessation for 3 and 13 weeks on INH pharmacokinetics were investigated in this open-label, randomized, crossover study.

Materials and Methods: A total of 68 nondiabetic subjects were included in the study: 38 smokers (Sm) (21 cigarettes/d) and 30 nonsmokers (NSm). At baseline, all subjects received either INH (2 mg) or subcutaneous (SC) insulin (6U) in a randomized, crossover design. In Sm, the second (INH2) and third (INH3) doses of INH were administered 3 and 13 weeks after smoking cessation, respectively. Plasma carboxyhemoglobin and serum cotinine concentrations were used to measure smoking status.

Results: At baseline (INH1), Sm had significantly higher mean maximum insulin concentration (C_{max}) and area under the curve ($AUC_{0–360}$), and a significantly shorter time to mean maximum concentration (T_{max}) compared with NSm (72.3 vs 15.8 μU/mL; 4850 vs 1410 μU·min/mL; and 31 vs 53 min, respectively) (*P*-value <0.05 for all 3 variables). After 3 weeks cessation, C_{max} and $AUC_{0–360}$ were significantly reduced to 35.7 μU/mL and 2850 μU·min/mL (*P*-value <0.05 vs INH1 for both variables), and T_{max} increased to 41 minutes. No further reductions were observed after 13 weeks cessation: C_{max} 43.1 μU/mL, $AUC_{0–360}$ 3260 μU·min/mL and T_{max} 40 minutes. Sm had a higher rate of hypoglycemia versus NSm, but most events were mild. There were no serious adverse events or discontinuations due to safety concerns.

Conclusion: INH absorption was reduced by almost 50% after 3 weeks of smoking cessation. This reduction was maintained after 13 weeks cessation, but the bioavailability of INH still remained greater than that of NSm. These findings concur with previous investigations that absorption of INH and other small molecules is increased in Sm compared with NSm.

This study was funded by Pfizer Inc

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Effect of intercurrent respiratory tract infections on inhaled human insulin (Exubera®) therapy: a retrospective pooled analysis of controlled phase 2 and 3 trials

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Background and Aims: The availability of inhaled human insulin (INH; Exubera® (insulin human [rDNA origin]) Inhalation Powder) may increase patient acceptance of insulin, improve glycemic control and reduce the burden of diabetes. INH has been administered to over 1900 adult type 1 (T1DM) and type 2 (T2DM) diabetes patients in controlled clinical trials. Initial premeal INH doses were recommended based on patient body weight. Doses were subsequently adjusted using premeal fingerstick glucose values. The aim of this study was to assess the incidence and potential clinical implications of intercurrent respiratory tract infections (iRTIs*) on INH therapy.

Materials and Methods: Retrospective analysis of pooled data from 14 controlled Phase 2 and 3 clinical trials (3 to 24 months duration).

Results: Intercurrent RTIs were common in all treatment groups with ≤ 1.5% of events leading to permanent discontinuation (Table). There were no apparent changes in glycosylated hemoglobin levels (%) or overall hypoglycemic rates (events/subject-month) with iRTIs in any treatment group. In T1DM patients, the mean fasting plasma glucose (mg/dL) and rates of severe hypoglycemia (events/100 subject-month) increased during iRTIs in both INH (161.5 vs 170.6 [9.0 vs 9.5 mmol/L]; 3.77 vs 7.72, respectively) and SC groups (171.6 vs 175.8 [9.5 vs 9.8 mmol/L]; 4.43 vs 8.58, respectively); no changes were seen in T2DM patients.

Conclusion: In summary, INH is well tolerated, safe, and efficacious in adult diabetes patients during iRTIs. During iRTIs, close monitoring of blood glucose concentrations and dose adjustment may be required on an individual basis.

Table

	T1DM		T2DM		
	INH	SC	INH	SC	OAs
Evaluable subjects (n)	698	705	1277	488	644
Subjects with iRTIs* (%)	62.2	57.2	44.7	51.8	30.1
iRTIs (n)	1010	889	1123	501	328
iRTI leading to temporary study drug discontinuation (n; %)	19; 1.9	0; 0	22; 2.0	0; 0	0; 0
iRTI leading to permanent discontinuation (n; %)	6; 0.6	0; 0	17; 1.5	0; 0	0; 0

INH=inhaled human insulin; SC=subcutaneous insulin; OA=oral agents *iRTIs=COSTART preferred terms of all causality: bronchitis, laryngitis, pharyngitis, pneumonia, rhinitis, asthma, RTI, and/or sinusitis.

This study was funded by Pfizer Inc

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Can the human insulin inhalation powder (HIIP) delivery system be used during an upper respiratory infection (URI)? The effect of URI on the pharmacokinetics (PK) and glucodynamics (GD) of HIIP in healthy subjects

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Background and Aims: Inhaled insulin is being tested as an alternative to subcutaneous (sc) injectable insulin. One concern is the potential for URIs to affect the delivery of dry powder insulin, potentially causing changes in both its bioavailability and variability. This may result in inconsistent blood glucose control, causing either hyper- or hypoglycaemia. Our study assessed the effect of symptomatic rhinovirus 16 (RV16) infections on the PK, GD, and safety and tolerability of HIIP delivered to healthy nondiabetic patients using the Lilly/Alkermes HIIP delivery system.

Materials and Methods: Twenty-one healthy, non-smoking male and female subjects (age 20.3±2.1 years; BMI 22.8±1.7 kg/m^2) participated in a single-centre, single-sequence, 2-period, euglycaemic glucose clamp study. Subjects received 1 dose of HIIP equivalent to 12U of sc insulin (5.2 mg), and underwent a clamp procedure. On a different day, subjects received a nasal inoculation with the RV16 virus. Two to 4 days later, symptomatic subjects received a second dose of HIIP (equivalent to 12U) and underwent a second clamp. Blood samples were collected to determine serum insulin immunoreactive concentrations for PK analysis. Glucose infusion rates and times were recorded for GD analysis.

Results: Sixteen subjects completed both doses/clamps. Pharmacokinetic and GD parameters, before or during infection, were comparable as shown in the table. The variability of PK and GD parameters did not increase during URI as indicated by the CV%. There were no serious adverse events reported, and no subjects had difficulty using the system. No significant change in mean FEV1 and FVC was observed following HIIP administration or during infection.

Parameter (Unit)	RV16 Infection Geometric LS Mean (CV%)		Ratio (%) [90% CI]
	Before (n=21)	During (n=16)	
$AUC_{(0\text{-}t')}$ (pmol•min/L)	46300 (51)	52600 (32)	114 [96, 135]
C_{max}(pmol/L)	248 (45)	260 (36)	105 [89, 124]
G_{tot}(mg)	61800 (104)*	68700 (80)	111 [89, 138]
R_{max} (mg/min)	327 (84)*	325 (50)	99 [77, 128]

*n=19

$AUC_{(0\text{-}t')}$: the area under the serum IRI concentration curve from time of insulin administration to time insulin level returns to baseline; C_{max}: maximal serum IRI concentration; CV: coefficient of variation; $G_{tot(0–600)}$ amount of glucose infused from time of insulin administration to 600 min; LS mean: least squares mean; n: number of subjects; R_{max}: maximal glucose infusion rate; CI, confidence interval.

Conclusion: These findings suggest that the presence of URI does not significantly alter the PK and GD of insulin delivered by the Lilly/Alkermes HIIP delivery system. Further, HIIP was safe and well-tolerated in subjects during URI. Patients should be able to continue to use this delivery system during a moderate URI.

This study was funded by Eli Lilly and Company

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Dose interchangeability between human insulin inhalation powder (HIIP) 2U- and 6U-equivalent Capsules

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Background and Aims: The Lilly/Alkermes inhaled insulin delivery system is designed to be patient- and provider-friendly, and to include the ability to freely exchange dosage strengths as needed.
Material and Methods: A phase I, open-label, randomised, dose-escalation, 5 period complete block design, euglycaemic glucose clamp study was conducted in healthy, nonsmoking, male, Japanese subjects (n=16) at a single center. Among other objectives, the relationship between the administration of three 2U-equivalent (0.9 mg each) and one 6U-equivalent (2.6 mg) HIIP capsules delivered by Lilly/Alkermes HIIP delivery system, was evaluated based on insulin pharmacokinetic (PK) and glucodynamic (GD) parameters. Each subject was randomly assigned to 1 of 4 possible dosing sequences, and received four single doses of HIIP: one 6U capsule; two 6U capsules; three 6U capsules; three 2U capsules and one dose of subcutaneous insulin lispro (12 U, 0.4 mg). Serum immunoreactive insulin (IRI) concentrations were measured for PK evaluation using a validated radioimmunoassay. Glucodynamics were assessed based on glucose infusion rate data collected during the glucose clamp procedure for up to 10 hours.
Results: PK and GD parameters after administration of three 2U capsules and one 6U capsule and the ratios using the 6U capsule as reference, are listed in the table below. AUC represents the total exposure of HIIP, and C_{max} is the highest exposure. G_{tot} represents the total effect of HIIP, and R_{max} is the highest effect.

Parameter (unit)	Treatment	Geometric Mean	Ratio (3×2U/1×6U) and [90% CI]
$AUC_{(0\text{-}t')}$ (pmol·min/L)	3×2U	23355	0.93 [0.75, 1.14]
	1×6U	25142	
C_{max} (pmol/L)	3×2U	120	0.95 [0.82, 1.11]
	1×6U	126	
$G_{tot\ (0–600)}$ (g)	3×2U	29.6	1.01 [0.69, 1.49]
	1×6U	29.3	
R_{max} (mg/min)	3×2U	132	0.96 [0.79, 1.17]
	1×6U	137	

*$AUC_{(0\text{-}t')}$: the area under the serum IRI concentration curve from time of insulin administration to time of insulin level returns to baseline; C_{max}: maximal serum IRI concentration; $G_{tot(0–600)}$ amount of glucose infused from time of insulin administration to 600 min; R_{max}: maximal glucose infusion rate; CI, confidence interval.

The ratio of geometric mean between the two dose strengths for AUC(0-t') C_{max}, R_{max} and G_{tot} were close to 1 and the 90% confidence intervals (CI) included 1. Bioequivalence based on the (0.80–1.25) reference range for the 90% CI could only be statistically concluded for C_{max}.

Conclusion: Given the typical variability in PK and GD after insulin administration, and the fact that the study was not specifically powered to detect the bioequivalence between these two dose strengths, patients should be able to use three 2U-equivalent capsules and one 6U-equivalent capsule interchangeably in the clinical setting.
This study was funded by Eli Lilly and Company

1010

The availability of inhaled human insulin may lead to a greater acceptance of insulin therapy in European type 2 diabetes patients failing on diet and/or oral agent therapy

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Background and Aims: Despite the proven benefits of intensive glycemic control, over 50% of patients with type 2 diabetes mellitus (T2DM) suboptimally controlled on oral agents (OAs) delay initiating subcutaneous (SC) insulin therapy for at least 4 to 6 years. Furthermore, a Canadian study found that insulin-naïve T2DM patients appear willing to sacrifice adequate glycemic control to reduce the number of daily insulin injections − a disconcerting phenomenon given the rapidly increasing prevalence of T2DM worldwide and increasing burden of diabetes-related complications on healthcare resources. We recently reported that the theoretical availability of inhaled human insulin (INH, Exubera® (insulin human [rDNA origin]) Inhalation Powder) significantly increased the proportion of T2DM patients who accept insulin therapy. The aim of this substudy was to examine the potential impact of INH availability on the T2DM patient acceptance of insulin therapy in the European setting, where insulin is predominantly administered with a pen.
Materials and Methods: T2DM patients failing on diet and/or OAs ($HbA_{1c} \geq 8\%$) were randomized into 2 groups. Both groups (A and B) received educational information on currently licensed treatment options (including OAs and SC insulin); Group B received the same information with the exception that INH was offered as an additional treatment option. Subjects were then asked to make a theoretical choice of therapy, including no change in their current treatment. Patients consulted with their physician/diabetes

nurse on treatment options and completed questionnaires concerning their choice. The primary outcome was the proportion of T2DM patients choosing insulin.
Results: In total, 576 T2DM patients (A=288, B=288) were recruited in 5 European countries (France, Germany, Italy, Spain, and Sweden). In Group B, 127 patients (44%) chose a treatment option that included insulin compared with 48 (17%) in Group A (odds ratio 3.9, 95% CI [2.7, 5.8]; $P<0.0001$). In Group B, INH was chosen by 101 patients (35%) whereas 26 (9%) patients chose SC insulin. Despite a mean HbA_{1c} of 9.1% and the availability of insulin pens, significantly more patients in Group A (132 [46%]) chose to make no change to their therapy compared to patients in Group B patients (86 [30%]; odds ratio 0.50, 95% CI [0.36, 0.71], $P<0.0001$). In a subgroup of T2DM patients uncontrolled on 2 OAs − those most likely to require insulin to achieve glycemic control − 83 patients (50%) in Group B chose a treatment option that included insulin compared with 32 (21%) of those in Group A. No systematic differences between countries were found.
Conclusion: In European T2DM patients failing to achieve target glycemic control with diet and/or OA therapy, the availability of INH as a potential treatment option encourages more patients to accept insulin therapy than SC insulin, even where insulin pens are predominantly used. The availability of INH could result in increased insulin acceptance by European T2DM patients, which would lead to improved glycemic control and better short- and long-term health outcomes.
Supported by Pfizer Inc

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Pharmacodynamics of new insulin

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Expected additive effects of combining insulin detemir and human insulin on glucose metabolism in rats
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Background and Aims: Insulin detemir (IDet) is a long-acting acylated desB30 human insulin designed to cover basal insulin requirements in diabetic patients. Co-administration of short acting insulin at mealtime is frequently used to obtain adequate glycemic control.
Materials and Methods: To study the clinical relevant pharmacodynamic interactions between human insulin (HI) and IDet on glucose disposal *in vivo*, the two insulins were tested separately and in combinations during steady state hyperinsulinemic-euglycemic clamp conditions in Sprague Dawley (SD) rats instrumented with permanet carotic and jugular chateters. HI and/or IDet was infused intravenously at a constant rate for 5 hours into conscious, unrestricted rats and the mean glucose infusion rates (GIR) during the last hour of the clamp was used as measure of efficacy. Three pair-wise equi-efficacious doses (1D, 2D, 4D) of HI (1.5, 3, 6 mIU/kg/min) and IDet (2.5, 5, 10 mU/kg/min) and two HI+IDet combination doses (1D+1D and 2D+2D) were tested. Furthermore, lipogenesis via ^{3}H-glucose was measured in primary rat adipocytes incubated for 2 hours at 37 Celsius with mixed concentrations of HI and IDet.
Results: Clamp data are presented in the table.

Steady state GIR (mg/kg/min).

Dose	HI	ID	HI+IDet (1D+1D)	HI+IDet (2D+2D)
1D	9.6±1.9	5.7±1.0	-	-
2D	17.5±1.6	15.9±2.3	16.6±1.9	-
4D	29.4±2.3	25.7±1.6	-	27.0±2.0

The lipogenic response *in vitro* showed full agonism of IDet compared to HI, and the interaction of the two ligands was primarily additive with a trend towards synergy.
Conclusion: The effects of combining IDet and HI are additive on glucose disposal *in vivo* and and on lipogenesis *in vitro* as expected for drugs acting via the same receptor.

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WITHDRAWN

1013

Equivalent metabolic effects of insulin detemir and human insulin on target tissues during hyperinsulinaemic -euglycaemic clamp conditions in Zucker obese rats
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Background and Aims: Insulin detemir (IDet) is a long-acting acylated desB30 human insulin designed to cover basal insulin requirements in diabetic patients. Theoretically, the effects of IDet on glucose metabolism in various insulin sensitive tissues may differ from that of human insulin (HI).
Materials and Methods: To investigate possible differential effects, we undertook series of 5 hour intravenous hyperinsulinemic-euglycemic clamp studies in conscious

Zucker obese rats instrumented with permanent carotic and jugular catheters. Whole body glucose disposal assessed by the glucose infusion rates (GIR) to maintain euglycemia and ^{3}H-glucose-determined rates of endogenous glucose production (EGP) and glucose utilization (GU) were compared at lower, medium and higher doses (LD, MD, HD) of IDet (10, 20, 40 mU/kg/min) and HI (5, 10, 20 mIU/kg/min) (table 1). In a separate clamp study (table 2), ^{14}C-2-deoxyglucose (2DG) uptake was measured in white/red gastrocnemius (WGM/RGM) and soleus (SM) muscle and in subcutaneous/epididymal fat (SCF/EPF) tissue at HI and IDet doses resulting in matched rates of GU (10 mIU/kg/min and 25 mU/kg/min, respectively). Data given as mean±SE for n=5–6/group.

Results:

Table 1. Glucose turnover (mg/kg/min).

	$IDet_{LD}$	$IDet_{MD}$	$IDet_{HD}$	HI_{LD}	HI_{MD}	HI_{HD}
GIR	3.9±1.2	5.7±0.7	15.1±0.8	3.7±0.5	8.3±1.3	13.5±1.4
Delta-EGP	−1.7±1.0	0.0±0.6	−5.0±1.6	−1.3±0.9	−3.8±0.5	−4.0±0.9
Delta-GU	2.2±2.0	5.4±1.2	9.7±1.0	2.4±1.5	4.7±1.4	9.5±1.0

Table 2. GU and tissue specific 2DG uptake (mg/kg/min).

	Clamp GU	WGM	RGM	SM	SCF	EPF
IDet	18.8±1.0	6.5±1.7	23.5±5.8	58.2±14.1	4.6±0.4	3.7±0.5
HI	18.8±0.8	11.7±3.1	31.9±3.5	68.0±8.0	4.4±0.3	3.4±0.3

Conclusion: IDet has similar effects compared to HI on suppression of EGP and stimulation of GU. Furthermore, at matched rates of whole body GU, IDet stimulates 2DG uptake in various muscle and fat tissues to the same extent as HI. These findings indicate that IDet and HI have equivalent effects on glucose metabolism *in vivo*.

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Intra-dermal insulin lispro application with a new microneedle delivery system led to a substantially more rapid insulin absorption than subcutaneous injection

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Background and Aims: We investigated the pharmacokinetic and pharmacodynamic effects of insulin Lispro applied intra-dermally with a new microneedle delivery system. Effective and strictly intra-dermally administration was achieved by the special design and shortness of a needle as part of the system.

Materials and Methods: Ten healthy male volunteers (age 31±6 years, BMI 23.7±1.7 kg/m^2 (mean±SD)) received 10 U insulin Lispro s.c. on one study day and the same dose intra-dermally via 3 different microneedles lengths: 1.25, 1.5 and 1.75 mm under euglycemic glucose clamp conditions (target level 5.0 mmol/L, continuous i.v insulin infusion of 0.15 mU/kg/min, clamp duration 360 min post-dosing).

Results: Intra-dermally applied LIS was absorbed more rapidly compared to sc application as indicated by lower values for $t_{INS\text{-}max}$ (1.25 mm: 40, 1.5 mm: 36, 1.75 mm: 47 min vs. sc 56 min; $p<0.05$) and $AUC_{INS\text{-}0\text{-}1\ h}$ (3752, 3841, 3817 vs. 2546 h*μU/mL; $p<0.05$). The PD measures confirm the more rapid onset of action with intra-dermal injection ($t_{GIR\text{-}max}$ 28, 29, 21 vs. 41 min; $p<0.05$), but with a comparable maximal metabolic effect (GIR_{max} 9.4, 8.9, 8.6 vs. 9.6 mg/kg/min; NS). In contrast to other alternative routes of insulin delivery, the relative bioavailability (147, 155, 150%) and the relative bioeffect (142, 137, 124%) of intra-dermally applied insulin was higher than with sc. There were no local skin reactions at any of the injection sites.

Conclusion: Intra-dermal injection of insulin Lispro by means of the microneedle system studied is a safe and effective method of delivering insulin rapidly and completely. Because of the rapid onset of the metabolic effect this application form appears to offer a great potential for prandial insulin administration.

Supported by Becton Dickinson

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Viaject™ insulin has faster onset of action and less intra-individual variability than regular human insulin in patients with type 1 diabetes

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Background and Aims: Viaject™ Insulin (VJ) is a formulation of recombinant human insulin and Generally Regarded as Safe excipients, with significantly more rapid absorption kinetics than regular human insulin (RI). We investigated the pharmacodynamic (PD) and pharmacokinetic (PK) properties of VJ and RI by means of the glucose clamp technique. The aim was to evaluate the intra -subject

variability of the PD and PK properties of VJ and RI in patients with Type 1 diabetes.

Materials and Methods: Fourteen patients with Type 1 diabetes participated in six, 10-hour euglycaemic glucose clamps (blood glucose target 90 mg/dl). During a 2 hour run in before dosing, the target level was achieved by means of a basal insulin infusion. The basal insulin infusion was shut off before dosing. Patients were 4 females and 10 males, age 35±11 years (mean±SD), and were randomly assigned to a sequence of 2 experimental blocks. Each block consisted of 3 doses 0.1 IU/kg of VJ or RI, respectively, administered by abdominal s.c. injection in this double-blind, cross-over study. The glucose clamps were continued for 8 hours after dosing or terminated when blood glucose exceeded 270 mg/dl. The time to maximal plasma insulin concentration (Tmax) and the time to maximal glucose infusion rate (GIR Tmax) were used as outcome measures. In addition the within-patient standard deviation was calculated for these parameters and compared with a paired two-tailed t-test.

Results: Pharmacodynamic and pharmacokinetic data of VJ and RI indicated that VJ has an earlier onset of action and shorter time to maximal plasma insulin concentration when compared to RI. The GIR Tmax was 94.4±44.9 min for VJ and 144.2±66.6 min for RI (p=0.01). Plasma insulin Tmax was 31.1±14.3 min for VJ and 112.3±48.9 min for RI, (p=0.00001). Additionally, the within subject variability of Tmax (p=0.04) and the within subject variability of GIR Tmax (p=0.015) was significantly less for VJ than for RI as measured by standard deviation. No clinically significant adverse events occurred.

Conclusion: Viaject™ is a novel formulation of recombinant human insulin that replicated its initial finding of significantly more rapid onset and faster absorption kinetics in patients with Type I diabetes. The results also show that VJ has significantly less intra-individual variability than RI.

Supported by Biodel Inc.

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Insulin glulisine: dose response relationship in subjects with type 1 diabetes

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Background and Aims: There is sparse information on the dose response relationship of insulin analogues in patients with diabetes. This randomized, complete crossover study in Type 1 diabetes (T1DM) subjects (n=18) assessed pharmacokinetic and pharmacodynamic systemic insulin exposure and the metabolic effect of 0.075, 0.15 and 0.3 U.kg^{-1} of subcutaneously injected insulin glulisine (GLU) or regular human insulin (RHI) during a BIOSTATOR-based euglycaemic glucose clamp.

Materials and Methods: Bioequivalence (BE) criteria, with 0.15 U.kg^{-1} as reference dose, were applied for dose-proportionality testing in insulin exposure and glucose utilization.

Results: Insulin overall exposure (INS-AUC_{total}) and metabolic effect (GIR-AUC_{total}) were comparable for each dose of GLU and RHI. However, GLU was twice as rapidly absorbed (INS-T_{max}; $p<0.05$) and had a higher metabolic effect during the 2 hours post-injection (GIR-AUC_{2h}; $p<0.05$) versus RHI. GLU and RHI showed a dose-proportional increase in overall insulin exposure (INS-AUC_{total}) and maximum insulin (C_{max}) for each dose. The overall metabolic effect (GIR-AUC_{total}) was proportional for 0.075 and 0.15 U.kg^{-1} of GLU. Dose-separation was observed for any subject with GLU and RHI, with regards to INS-AUC_{total}, C_{max} and essentially in GIR-AUC_{total}; however, none was observed for the metabolic effect during the 2 hours post-injection (GIR-$AUC_{2\ h}$). Absorption (INS-$T_{90\%}$) and activity (GIR-$T_{90\%}$) completion were ~120 minutes less for GLU than RHI; doubling the dose added ~60 minutes to the action of both insulins.

Conclusions: In T1DM subjects, GLU displays dose-proportionality in insulin exposure from 0.075–0.3 U.kg^{-1}, which is associated with dose-proportional metabolic activity for 0.075 and 0.15 U.kg^{-1}.

	GLU			RHI		
Dose (U.kg^{-1})	0.075	0.15	0.3	0.075	0.15	0.3
C_{max} (μU.mL^{-1})	42*	72	140**	22**	38	69*
INS-T_{max} (min)	47	57	72	82	104	119
INS-$AUC_{2\ h}$ (μU.min.mL^{-1})	3792**	6676	12992**	2211	3448	5792*
INS-AUC_{total} (μU.min.mL^{-1})	5341**	11196	24891**	5969**	10608	21226**
INS-$T_{90\%}$ (min)	149	205	242	313	348	410
GIR_{max} (mg.$kg^{-1}min^{-1}$)	4.1	6.4	6.7	2.5*	4.6	5.4
GIR-T_{max} (min)	87	114	121	178	169	207
GIR-AUC_{2h} (mg.kg^{-1})	314	491	536	127	219	294
GIR-AUC_{total} (mg.kg^{-1})	499*	1090	1476	416	1076	1555
GIR-$T_{90\%}$ (min)	180	238	275	278	330	423

*Point estimate and upper or lower limit within BE limits; **dose-proportionality within BE limits with reference to 0.15 U/kg dose

Supported by sanofi-aventis

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Variability in concentration and glucodynamic profiles of insulin glargine in healthy volunteers

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Background and Aims: Traditional long-acting insulin preparations, such as NPH insulin and insulin ultralente, are associated with a peak-action profile and large day-to-day variability in absorption following administration, which can result in periods of unwanted hypo- or hyperglycaemia. Insulin glargine has a predictable flat time-action profile with lower intra-subject variability in comparison to the above mentioned insulin formulations in systemic insulin exposure and glucodynamic effects.
Materials and Methods: Using the Biostator supported euglycaemic clamp technique, two formulations of insulin glargine (reference: R; Test: T) were tested for equivalence in exposure (insulin [INS]) and efficacy (glucose infusion rate [GIR]) over 30 hours in 24 healthy male volunteers (mean age 31 years [range 20–48 years]; BMI 24 kg/m^2 [20–27 kg/m^2]). Subjects received replicate single subcutaneous injections of insulin 0.4 IU/kg body weight in a four-way complete crossover trial, randomized for RTRT or TRTR sequences.
Results: Both R and T produced flat INS and GIR profiles, superimposable within and between preparations, establishing equivalence (T/R ratio within 80–125%) in INS- $AUC_{0–30\ h}$ and GIR-$AUC_{0–30\ h}$, and secondary metrics (INS-C_{max}, GIR_{max}). Overall, intra-subject variabilities in INS-AUC, assessed as coefficient of variation (CV %) and derived from mean square error terms of ANOVA were 18%; the intra-subject variabilities for GIR-AUC values was 32%. Individual variability in INS-AUC did not completely correspond to individual variability in GIR-AUC, highlighting the greater variability in glucodynamic efficacy in general. See Table.

	Reference R	Test T	Confidence interval T/R (95% CI)
INS-$AUC_{0\text{-}end}$ (μIU.h.ml^{-1})	425	414	97 (92; 103)
CV % (INS-$AUC_{0–30h}$) [*]	13	19	146 (117; 213)
GIR-$AUC_{0–30h}$ (mg.kg^{-1})	2743	2796	102 (91; 115)
CV % (GIR-$AUC_{0–30h}$) [+]	34	30	88 (75; 96)
	R & T Combined		**R & T (95% CI)**
CV % (INS-$AUC_{0\text{-}30h}$) [*]	18		13; 22
CV % (GIR-$AUC_{0–30h}$) [+]	32		23; 41

Chinchilli & Esinhardt on ln-transformed [*] or raw data [+]

Conclusions: This study establishes equivalence of the two insulin glargine formulations, the superimposable flat concentration and glucodynamic profiles of insulin glargine, and attests to the low intra-subject variability of insulin glargine.
Supported by sanofi-aventis

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Pharmacokinetics and -dynamics of therapeutic doses of the "long-acting" insulin analogues glargine and detemir at steady-state in type 1 diabetes mellitus

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To compare pharmacokinetics and -dynamics (PD) of the "long-acting" insulin analogues glargine (GLA) and detemir (DET), 24 subjects with T1DM naïve to GLA and DET were studied twice (randomized, double-blind, cross-over study). 14 subjects completed, 39±13 years, BMI 22±1.7, kg/m^2, HbA_{1c} 7.0±0.4% (means±SD). Plasma glucose (PG) was clamped at target of 5.5 mmol for 24-h after sc injection of 0.35 UI/kg GLA or 0.35 U/Kg DET in the abdomen at 19:00 h after 2 week treatment with either GLA or DET (o.d. at 19:00 h). Primary endpoints were *onset of action* (time at which iv G was initiated after sc GLA or DET); *minimal duration of action* (time at which PG was > 6.5 mmol/l in absence of G infusion). Results are given referred to insulin A and B (data locked). With A, PG remained at 5.7±0.2 mmol/l until 24 h (all 14 subjects completed study). With B, PG started to increase after 11 h and was 10.5±2.17 mmol/l by 24 h (only 2 out of 14 subjects completed study because PG increased >11.1 mmol/l before 24 h). G infusion rate (GIR) was constant over 24 h with A, but decreased markedly with B. Total activity (GIR-$AUC_{0\text{-}end\ of\ GIR}$) was 1623±792 and 832±252 mg/Kg, whereas the end of GIR was 23.4±1.4 and 15±4.2 h for insulin A and B, respectively ($p<0.01$). Other pD and clinical endpoints are shown below:

	Onset of action (h)	Minimal duration of action (h)	GIR (0–12 h) (mg/Kg/min)	GIR (12–24) (mg/kg/min)	GIR max (mg/kg/min)	GIR Tmax (h)
Insulin A	1.5±0.7	24±0.6	1.2±0.2	1.1±0.2	2.0±0.9	10.6±7.0
Insulin B	1.5±0.6	16.4±3.3*	1.0±0.1	0.2±0.1*	1.7±0.5	7.7±2.0

*$P<0.05$ vs A.

Conclusions: Insulin A (likely GLA) and B (likely DET) at steady-state, have different total activity despite identical nominal units, and different pD which should be taken into consideration for dosing, number and timing of daily administrations in T1DM.

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Genetics of complications

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Proliferative retinopathy clusters in type 1 diabetic siblings

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Background and Aims: Diabetic nephropathy clusters in families suggesting that genetic factors play a role in the pathogenesis of this complication. Whether proliferative retinopathy, a complication frequently seen in conjunction with nephropathy, also clusters in families is less clear. Therefore the aim was to study the presence and potential clustering of proliferative retinopathy (PDR) in type 1 diabetic sibpairs.
Materials and Methods: As part of the ongoing FinnDiane Study we have so far collected all available data on the development of diabetic retinopathy from almost 2000 patients including over 26 000 pages of ophthalmic records. In addition 23931 fundus photographs from 1438 patients have been scanned at 300 dpi and saved in our digital image library. The images have been graded using a modified ETDRS-scale by an ophthalmologist unaware of the demographic data and the presence or absence of other diabetic complications. The first 714 (400 M/314 F) completely classified patients were used in the present analysis.
Results: There were 70 sibpairs (140 patients) and in 12 of them (24 patients) both had PDR (17%). Out of all the 714 patients studied 242 patients had PDR (34%). In order to detect potential clustering of PDR, a group of control sibpairs was constructed from the available 218 age-matched unrelated PDR patients. The difference in the time interval from diagnosis of diabetes to onset of PDR within sibpairs was shorter in related (4.8±3.1 years) than unrelated (7.6±6.5 years) patients (p=0.01). This observation is suggestive of familial clustering of PDR and is further supported by the positive correlation between related sibpairs (r^2=0.352, p=0.04). The duration to incident PDR was similar in both groups, 20.6±7.3 years in the unrelated and 18.6±7.3 years in the related sibpairs (p=0.21). The sex distribution was equal between (p=0.41) and within (p=0.25) the related and unrelated sibpairs. Furthermore, the related and unrelated sibpairs were no different with regards to age at onset (p=0.31), HbA_{1c} (p=0.10), or blood pressure (p=0.98). Notably nephropathy status showed a remarkable concordance in related as compared to unrelated sibpairs (p=0.002).
Conclusion: PDR seems to cluster in related sibpairs suggesting that genetic factors may play a role in the pathogenesis of PDR.
Supported by Silmäsäätiö

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Polymorphisms in the 5′-flanking region of the PEDF gene are associated with diabetic retinopathy and diabetic macular oedema in type 2 diabetes

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Background and Aims: PEDF (pigment epithelium-derived factor), a member of the serpin superfamily of proteins, is an important neuromodulatory factor that can provide effective neuroprotection. In the retina, PEDF protects retinal neurons against insults such as oxidative stress and glutamate excitotoxicity. PEDF is also antiangiogenic and can inhibit the growth of blood vessels in the eye. Thus, PEDF is an attractive candidate gene for development of diabetic retinopathy (DR) and diabetic macular edema (ME).
Materials and Methods: In the present study, subsequent to the VEGF and eNOS genes, we examined three polymorphisms of the PEDF gene, C-811T, A-379G and Met72Thr. Among 416 Japanese patients with type 2 diabetes studied, 229 patients had no retinopathy, 101 had non-proliferative diabetic retinopathy (NPDR), and 86 had proliferative diabetic retinopathy (PDR). Macular edema was present in 17 patients with NPDR and 49 patients with PDR.
Results: Genotype and allele distribution of C-811T and A-379G, but not Met72Thr, were significantly different between patients with and without diabetic retinopathy. Logistic regression analysis revealed that patients with the C-811T genotype had an increased risk of developing DR (p=0.0002), with the −811C allele increasing the risk. The C-811T and A-379G polymorphism were in tight linkage

disequilibrium, and the A-379G genotype was also had an increased risk of DR (p=0.0003). Furthermore, the C-811T or A-379G genotype was a significant risk factor for ME, independently from severity of DR.
Conclusion: These results demonstrate that C-811T and A-379G polymorphisms of the PEDF gene is a novel genetic risk factor for DR and ME.

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EURAGEDIC study: analyses of 144 candidate genes for diabetic nephropathy

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Background and Aims: Diabetic nephropathy (DN) is likely to be a complex genetic trait. The objective of the EURAGEDIC study is to identify DN susceptibility genes. We report molecular genetic studies for 144 candidate genes for DN.
Materials and Methods: More than 4000 DNAs have been assembled from 3 European populations in France, Denmark and Finland including type 1 diabetic patients with (n=1057) and without (n=1127) DN for case/control study, and 532 parent-offspring trios with (n=244) or without (n=288) DN for transmission disequilibrium testing. Candidate genes were selected through assessment of linkage studies, knowledge of metabolic pathways, and animal models. After re-sequencing of the genes, 439 polymorphisms tagging the haplotypes with a frequency greater than 5% in at least one population or non-synonymous polymorphisms were identified and further sought for association with DN. Association was first tested in each population by single-point analysis and in the absence of heterogeneity across the 3 populations, a combined OR was provided. Any suggestive association (P<0.1) was checked for confirmation through TDT analysis in trios with DN and in the absence of heterogeneity across the two studies results were combined to provide an overall estimate of the OR.
Results: Fifty SNPs showed suggestive association with DN in single-point analysis, for 6 of which results were heterogeneous across the 3 populations. Results of the TDT-analysis for the 44 SNPs further analysed were not significant but were consistent with the results of the case/control study for 31 of them. Overall estimate of the OR for the combined analyses was significant at the 5% level for 13 SNPs in 10 genes: ACE_DI (OR=0.87 [0.78–0.98], p=0.018), ACE_17944CT (OR=1.17 [1.05–1.31], p=0.006), ATP1B2_3208GA (OR=1.20 [1.05–1.37], p=0.007), ATP1B2_3623CT (OR=0.88 [0.798–0.98], p=0.025), ENO2_8908CT (OR=0.85 [0.73–0.97], p=0.02), GLO1_20729AT (OR=1.20 [1.01–1.43], p=0.042), IGF2_rs4320932CT (OR=1.16 [1.02–1.32], p=0.021), NFATC1_133215TC (OR=1.18 [1.03–1.36], p=0.018), PRKWNK2_63213GA (OR=0.57 [0.34–0.97], p=0.036), UBA52_2351CT (OR=0.89 [0.80–0.98], p=0.025), VEGF_7541CA (OR=0.89 [0.79–1.00], p=0.046), TIMP1_2544GA (OR=0.867 [0.76–0.98], p=0.022), TIMP1_4418TC (OR=0.85 [0.76–0.95], p=0.006). Haplotype analysis as well as gene-gene interaction analysis for the whole dataset are still ongoing.
Conclusion: We found suggestive association between DN and markers in 10 genes. Further investigations are needed to clarify the role of those genes in the pathogenesis of diabetic nephropathy.
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Combinational effect of the genes encoding components of renin-angiotensin system on conferring susceptibility to diabetic nephropathy in Japanese subjects with type 2 diabetes

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Background and Aims: To evaluate a role of renin-angiotensin system (RAS) in conferring susceptibility to diabetic nephropathy, we examined the association between polymorphisms within the genes encoding angiotensin converting enzyme (ACE), angiotensinogen (AGT), angiotensin II receptor type 1 (AGTR1) and diabetic nephropathy in a large cohort of Japanese type 2 diabetic patients.
Materials and Methods: Single nucleotide polymorphisms (SNPs) within the ACE, AGT, and AGTR1 genes were selected from Japanese SNP database, and analyzed by

invader assay in 747 nephropathy cases and 557 control subjects.

Results: Ten SNPs within ACE gene were successfully genotyped, and 8 SNPs were significantly associated with diabetic nephropathy (P<0.05). Among them, 5SNPs were almost in complete linkage disequilibrium to the I/D polymorphism (Δ=1), and the allele corresponding to the D allele for ACE I/D polymorphism was considered to be a risk allele for diabetic nephropathy in this population (P=0.01, Odds ratio=1.34 95% CI: 1.07–1.69). Regarding the AGT gene, six SNPs including M235T polymorphism could be determined, and M235T and two other SNPs were significantly associated with diabetic nephropathy (M235T: P=0.01, Odds ratio=0.74, 95% CI: 0.59–0.94). In the AGTR1 gene, twenty-six SNPs could be genotyped, and 1SNP within the second intron was associated with nephropathy (P=0.029). We further examined the combinational effect of these genes by a step-wise logistic regression analysis. The result indicated that each gene might contribute to the susceptibility to diabetic nephropathy independently, and there was no significant synergistic interaction among these genes, but a significant additional effect of these three genes (final empirical P value= 0.00005). Subsequent logistic regression analysis revealed that the increased number of risk alleles (corresponding allele of D allele, C allele for rs2242638 in the ACE, M allele for the AGT, and C allele of rs388915 for the AGTR1) was an independent risk for the development and progression of diabetic nephropathy (P=0.0017, OR=1.42, 95% CI; 1.14–1.76). We could not find any SNPs within the RAS gene associated with hypertension in this cross sectional study.

Conclusion: We concluded RAS gene polymorphisms might contribute to the susceptibility to diabetic nephropathy in type 2 diabetes, and the combination of these genes is useful for the prediction of the development and progression of the disease.

Result of logistic regression analysis

	Sex (male 1, female 2)	Age	BMI	Duration of diabetes	HbA1c	Hyper-tension	Number of risk genotype
regression coefficient	0.821	−0.040	0.016	0.060	0.011	2.169	0.348
standard error	0.177	0.008	0.024	0.010	0.031	0.215	0.111
P	<0.0001	<0.0001	0.51	<0.0001	0.73	<0.0001	0.0017

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Role of genetic variability in antioxidant and DNA reparation enzymes in diabetic nephropathy

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Background and Aims: Diabetic nephropathy (DN) develops as a result of hemodynamic and metabolic changes accompanying diabetes. Hyperglycemia-induced overproduction of reactive oxygen species (ROS) is an important pathogenic determinant of DN. (In)efficient cellular protection by antioxidant enzymes is reflected in the overall extent of oxidative damage. Genetic variability in genes encoding antioxidant enzymes belong to functional candidates potentially modulating susceptibility to DN. Subsequently, oxidative DNA damage due to ROS overproduction activates DNA reparation. Unfortunately, this further aggravates cellular dysfunction/damage due to consumption of energy substrates and metabolic cofactors. The aim of the study was to investigate relationship between genetic variability in genes encoding poly-(ADP-ribose) polymerase (ADPRT), manganese and extracellular superoxide dismutase (SOD2, EC-SOD), catalase (CAT), glutathion peroxidase (GPx), glutathione S-transferases (GST) and susceptibility to DN.

Materials and Methods: Case-control study comprised a total of 421 unrelated Caucasian subjects, 236 type 2 diabetics with DN and 185 type 2 diabetics without DN. DN was classified according to the albumin excretion rate and glomerular filtration rate; diabetics with minimally persistent proteinuria were classified as DN. Genotypes of ADPRT C410T, C1362T and G1672A, SOD2 T245C, EC-SOD C760G, GSTM1 deletion, GSTP1 deletion, CAT -262C/T and GPx C593T were detected by PCR-based methodology. Differences in allele frequencies were tested by Fisher-exact test. Differences in genotype distributions and Hardy-Weinberg equilibrium were tested by $\chi 2$ test. Estimation of haplotype frequencies was performed in silico by PHASE software and differences in haplotype distributions tested by permutation test. Bonferroni correction was used to correct for multiple comparisons.

Results: No significant differences in allele, genotype or haplotype distribution of any of the SNPs studied were found between groups of subjects with and without DN (P>0.05). Solely the frequency of CAT-262C/T protective allele T was marginally significantly higher (P=0.06) in diabetics without DN.

Conclusion: Our results suggest that despite of pathophysiological importance of antioxidant defensive system its genetic variability does not constitute major genetic risk for the development of diabetic nephropathy in type 2 diabetics, at least in Czech population.
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Evidence for association of the NPY Leu7Pro polymorphism with type 1 diabetes and diabetic nephropathy in Swedish Caucasians

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Background and Aims: Neuropeptide Y (NPY) is present in the central and peripheral nervous system, several organs and plasma. NPY has been shown to regulate renal blood flow. A recent report indicates that the Leu7Pro (T1128C) polymorphism in this gene confers the susceptibility risk to diabetic nephropathy and coronary heart disease (CHD) in Finnish type 1 diabetic (T1D) patients (Pettersson-Fernholm et al. 2004). The present study evaluates whether this polymorphism is associated with T1D and diabetic nephropathy among Swedish Caucasians.
Materials and Methods: Genotyping of the NPY Leu7Pro polymorphism was performed by using dynamic allele specific hybridization (DASH). A total of 423 (female 230/male 193) T1D patients and 187 (116/71) non-diabetic control subjects were included. Among the patients, 174 (98/76) had diabetic nephropathy.
Results: The risk C allele was strongly associated with the development of T1D (P=0.042, OR=2.323 95% CI 1.028–5.246) and diabetic nephropathy (P=0.011, OR=2.614 95% CI 1.249–5.467) in women, but no significant association was seen in men.
Conclusion: The present study provides evidence that the NPY Leu7Pro polymorphism confers a significant genetic risk to the development of both T1D and diabetic nephropathy in Swedish women.
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Cardiovascular disease – clinical interventions

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Complications and cardiovascular risk factors in people with type 1 diabetes in the International Diabetes Management Practices Study (IDMPS)

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Background and Aims: Preliminary data from the IDMPS were used to assess the frequency of chronic complications and associated cardiovascular risk factors in people with type 1 diabetes (≥18 years) from 11 countries in Eastern Europe, Asia, and Latin America.
Materials and Methods: This observational study enrolls patients seen in current medical practice by physicians managing diabetic patients.
Results: Data are currently available for 1327 people (51% men) with type 1 diabetes who had a mean age of 36±13 years and a mean disease duration of 11 years. 18% were smokers. Mean HbA_{1c} was 8.2±1.96% and 27% of people had an HbA_{1c} <7%. 98% of people had microvascular complications (56% retinopathy, 45% nephropathy, 72% neuropathy) and 19% had macrovascular complications (10% coronary artery disease, 3% cerebrovascular disease, 11% peripheral vascular disease). Mean systolic/diastolic blood pressure (BP) was 122/75 mmHg. Antihypertensive medication was taken by 20% of people and 47% had achieved a BP below 130/80 mmHg. Target treatment values were achieved for LDL (<100 mg/dL) in 41% of people (11% receiving lipid lowering treatment), for HDL (>40 mg/dL) in 81% of people (18% receiving lipid lowering treatment), and for triglycerides (<150 mg/dL) in

72% of people (10% receiving lipid lowering treatment). Only 3% of people reached all 3 recommended targets for HbA_{1c}, BP and lipids.

Conclusion: Thus, although patients have wide access to medications that specifically treat these conditions, it appears that more suitable guidance and education are necessary to ensure their effective use by people with type 1 diabetes world wide.

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Integration of diabetes care improves general risk factor control in patients with type 2 diabetes attending comprehensive cardiac rehabilitation - the randomised DANSUK study

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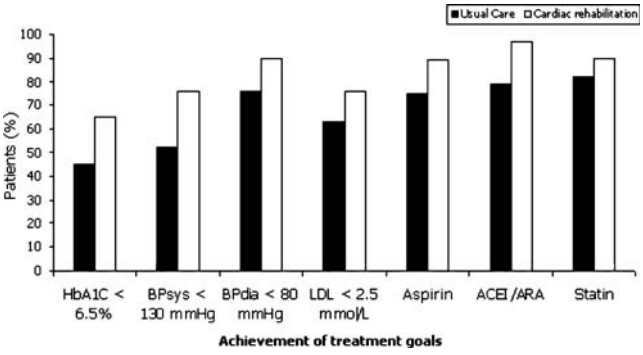
Background and Aims: Mortality and readmission rates for ischemic events are unacceptable high in patients with type 2 diabetes (T2DM). The DANSUK study was designed to test if integration of diabetes care in the settings of hospital-based comprehensive cardiac rehabilitation (CCR), would result in a further improvement of risk factor control in patients with T2DM compared to usual care (UC).

Materials and Methods: 201 patients eligible for CCR were randomized after being discharged from a department of cardiology. Thirty-four percent in each study group had T2DM. Patients allocated to UC received treatment in specialized outpatient clinics. Patients randomized to CCR received 6 weeks of exercise training, psychosocial support, patient education and intensified systematic risk factor control after 3, 6 and 12 months.

Results: After one year, the mean change of HbA1c was −0.7±0.9% (from mean 7.13±1.2% to mean 6.48±0.8%) in the CCR group compared to a mean change of −0.1±0.7% (from mean 6.95±1.1 to mean 6.87±1.2%) in the UC group ($p<0.05$). Systolic and diastolic blood pressure was improved 5%/7% in the CCR group (from mean 132±21/75±10 mmHg to mean 125±16/70±7 mmHg) compared to an improvement of 1%/1% in UC (from mean 131±21/72±9 mmHg to mean 129±23/71±11 mmHg)($p<0.01$). Insulin resistance was improved 7% in the CCR group compared to a deterioration of 15% in the UC group ($p<0.05$). Sixty-six percent of the patients in the CCR group obtained a treatment goal of HbA1c below 6.5% and more patients attending CCR were in treatment with cardio-protective agents at the end of the study ($p<0.05$).

Conclusion: In patients with T2DM, intensified CCR with an integrated diabetes module was more efficient in further improving risk factor control than UC. Diabetes care should be considered a core component in future settings of CCR.



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Three years follow-up of screen detected diabetic and non-diabetic subjects: who is better off? The ADDITION Netherlands study

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Background and Aims: As part of the ADDITION study *(Anglo-Danish-Dutch Study in general practice of intensive treatment and complication prevention in type 2 diabetic patients identified by screening)*, we performed a stepwise population-based screening in 2002–2004 in 79 general practices in the Netherlands. The first step of the screening consisted of a cardiovascular risk questionnaire. All subjects who had an elevated score (>6 points, range 0–29) on the questionnaire were invited for further diagnostic testing. Screen detected type 2 diabetic patients underwent subsequent treatment for cardiovascular risk factors but we do not know whether this also applies for screened non-

diabetic subjects with an elevated risk score. Our aim was to determine the risk of cardiovascular disease (CVD) and the extent of health care use of screened non-diabetic subjects with an elevated cardiovascular risk score compared to screen detected diabetic patients after three years of follow-up.

Materials and Methods: For the present study, 64 subjects with type 2 diabetes, 62 with impaired glucose tolerance (IGT), 86 with impaired fasting glucose (IFG) and 142 subjects with normal glucose tolerance (NGT), from 24 practices, screened from June to October 2002, were compared with regard to development of cardiovascular events, health care consumption, use of medication, and biochemical and antropometric parameters after three years follow-up.

Results: In total 16.1% of subjects with IGT, 9.3% of those with IFG and 4.2% of subjects with NGT developed diabetes during the follow-up period. Most cardiovascular events occurred in subjects with IFG (32.6 per 1,000 person-years), least in diabetic patients (15.7 per 1,000 person-years). The number of visits to care providers in the follow-up period was significantly higher in people with diabetes (33.5; interquartile range 22.3–46.0) compared to subjects with IGT (26.0; 15.0–43.5), IFG (23.0; 9.0–34.0) and NGT (20.0; 11.0–34.0). Also glucose, lipids, and blood pressure measurements were performed most frequently in diabetic patients. During follow-up, in all categories cardiovascular medication was prescribed more frequently with the strongest increase in diabetic patients. Blood pressure improved in all categories except NGT.

Conclusion: In three years of follow-up, screened non-diabetic subjects with an elevated risk score developed similar CVD risk as subjects with diabetes likely because of lacking adequate treatment for cardiovascular risk factors. These results underline that screened non-diabetic subjects with an elevated cardiovascular risk score should not be reassured only because of not having diabetes.

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Age at onset is an important factor of the severity of type 2 diabetes mellitus: CoDiC-analysis of clinical data obtained at multiple institutions across Japan

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Background and Aims: We now have the chance to treat type 2 diabetic (T2D) patients with a variety of anti-hyperglycemic drugs according to the patient's lifestyle and the underlying pathophysiology. However, it is difficult to get good glycemic control in many T2D patients and thus sufficiently protect diabetic complications. To clarify the factors for the severity of the disease, we analyzed clinical data in Japanese T2D patients treated with the drugs.

Materials and Methods: Clinical data were collected from 41 institutes across Japan, using the CoDiC® database:a diabetes data collection and management information system developed by The Japan Diabetes Clinical Data Management Study Group (JDDM). A total of 13,921 T2D patients treated with the drugs had been registered in 2004. The data were collected in the central analytical center. Analyses were carried out using Microsoft Excel and SPSS. Glycemic control was evaluated by hemoglobin A1c (HbA1c) which was measured using the HPLC method and the normal range was defined as 4.3–5.8%. The standardization of the HbA1c level was carried out in each institute according to the criteria recommended by the Committee of Japan Diabetes Society. The study protocol was approved by the JDDM ethics committee.

Results: In the patients, 37% were treated with monotherapy of oral hypoglycemic agent (OHA), 33% with combination therapy of OHAs, 11% with combination therapy of OHA plus insulin (INS) and 19% with monotherapy of INS. Patients with OHA plus INS and INS had a longer disease duration. Body mass index (BMI) was lower in patients with INS. Post-prandial plasma CPR was lower in the patients with OHA plus INS and markedly lower in the patients with INS. 28.3% of the patients had good glycemic control (HbA1c<6.5%), 49.8% had fair contol (6.5%<=HbA1c<8.0%) and 21.8% had poor control (HbA1c>=8.0%). Clinical data were analysed between the patients with good control and the patients with poor control. Age at onset was younger in the patients with poor control than that in the patients with good control (47.2 years vs 53.6 years, $p<0.000$) and also was same in the cases of patients with OHA, OHAs and INS, respectively. The age was the youngest in the patients who received intensive conventional insulin treatment and had poor control (42.6 years). Duration of the disease was longer and BMI, blood pressure (BP), total cholesterol (T-Cho) and fasting IRI were higher in the patients with poor control than those in the patients with good control. Logistic regression analyses showed that odds ratio for glycemic control was strong for age at onset (0.969, 95% CI: 0.964–0.974, $p<0.000$) as well as other clinical characteristics: duration of the disease, BMI, BP and T-Cho.

Conclusion: Age at onset is an important factor of the severity of T2D patients. We have to start intensive anti-hyperglycemic treatment in T2D patients as possible as earlier. Insulin-sensitizing agents should be positively added in T2D patients diagnosed at a younger age, with

high fasting IRI level, high BMI, high BP and dyslipidemia. Insulin therapy should be actively introduced in T2D patients diagnosed at a younger age, with relatively low BMI and with low postprandial plasma CPR level. Also, this result led us to promote the research for the factors attribute to the earlier onset of T2D: genetic and environmental factors.

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Utility of amlodipine/atorvastatin single-pill therapy in patients with diabetes mellitus or metabolic syndrome: Results from the JEWEL programme

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Background and Aims: Patients with diabetes and/or metabolic syndrome (MetS) are at high risk of cardiovascular (CV) disease. The prevalence of concomitant hypertension (HTN) and dyslipidaemia (DYS) is high among patients with these conditions who often have difficulty in reaching their aggressive therapeutic goals. This analysis of the JEWEL studies evaluated the utility of amlodipine/atorvastatin single-pill therapy in patients with HTN/DYS and diabetes or MetS.

Materials and Methods: The JEWEL programme consisted of two 16-week, international, open-label, multicentre, titration-to-goal studies in patients with HTN and DYS. The 2 study designs differed mainly on country of enrolment (JEWEL I: UK and Canada; JEWEL II: 11 European countries) and country/region-specific BP and LDL-C targets. Eight dosage strengths of amlodipine/atorvastatin single-pill (5/10–10/80 mg) were administered and electively uptitrated to reach targets as necessary. This planned analysis included patients with diabetes or those with MetS, as defined by the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) or International Diabetes Foundation (IDF) criteria. The percentage of these patients attaining goals for BP (diabetic patients,<130/80 mm Hg [UK: 140/80 mm Hg]; non-diabetic patients,<140/90 mm Hg [UK: 150/90 mm Hg]) and LDL-C (2.5–4.5 mmol/L depending on risk level and country goal) at end point was assessed.

Results: Across JEWEL I and II, 2245 patients received study medication and 2219 were included in the intent-to-treat (ITT) population. Overall, 696 (31%) of these ITT patients had diabetes while 1056 (47%) were classified with MetS according to NCEP ATP III and 1648 (74%) patients by IDF criteria. At the end of the studies, 37.2% of diabetic patients reached both BP and LDL-C therapeutic goals, 42.2% reached their BP goal alone, and 83.2% reached LDL-C goal alone. This compared with 65.9%, 77.2%, and 83.0% respectively, for patients without diabetes. Over half of the patients with MetS (50.9% [NCEP ATP III]; 53.9% [IDF]) achieved both BP and LDL-C goals, whereas almost two-thirds of patients without MetS (62.3% [NCEP ATP III]; 65.3% [IDF]) achieved both targets. Amlodipine/atorvastatin single-pill therapy was well tolerated in all patients and most adverse events were mild or moderate in intensity.

Conclusion: An additional 592 patients (an increase of 56%) were classified with MetS when IDF criteria were used versus NCEP criteria. Single-pill amlodipine/atorvastatin was effective and safe for reducing BP and LDL-C to target levels in patients with diabetes and/or MetS. These high-risk patients traditionally have difficulty obtaining BP and LDL-C goals, therefore it is important to apply novel treatment strategies to improve their overall CV risk factor profile.

Supported by Pfizer Inc

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Secondary prevention of cardiovascular disease using Alphabet Strategy in type 2 diabetes

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Background and Aims: Diabetes mellitus predicts a poor prognosis following a cardiovascular event. There is now a large evidence base demonstrating the benefit of secondary prevention measures. The Alphabet Strategy is diabetes care based around the mnemonic: A - Advice regarding smoking cessation, ideal weight, exercise; B - Blood pressure aiming for<140/80 mmHg; C - Cholesterol aiming TC<5, LDL<3, HDL>1; D - Diabetes care aiming HbA1c <7%; E - yearly fundoscopic exam; F - yearly foot exam; G - Guardian drugs with respect to aspirin, ACE inhibitors, and lipid lowering therapy. We present data from the

POEM (Practice of Evidence-based Medicine) Audit database showing the impact of the Alphabet Strategy on secondary prevention in those with type 2 diabetes mellitus.

Materials and Methods: The POEM Audit database of 400 consecutive patients with diabetes mellitus contains diabetes and cardiovascular parameters at time 0 (T0, referral) and time follow-up (Tfu). Of these, 86 had established cardiovascular disease. Statistical analysis was performed using Student's paired t-test and chi-squared test.

Results: Mean age of patients with atherosclerotic disease at T0 was 64 years. 60% were male. Mean duration of diabetes was 9 years. Average follow-up through the POEM database was 5 years. Alphabet Strategy results T0 vs Tfu (excluding eye and foot exams):

- Advice: Smokers 12.8% vs 9.3% (p=0.33)
- Blood pressure: SBP 144 mmHg vs 139 mmHg (p=0.037); DBP 79 mmHg vs 74 mmHg (p<0.001)
- Cholesterol: TC 5.7 mmol/l vs 4.7 mmol/l (p<0.0001); HDL 1.01 mmol/l vs 1.20 mmol/l (p<0.0001)
- Diabetes control: HbA1c 7.7% vs 8.0% (p=0.15)
- Guardian drugs: Aspirin 68% vs 93%; ACEI or ARB 39% vs 88%; Lipid lowering therapy 29% vs 81% (of which 97% were statins)

Conclusion: Use of the Alphabet Strategy resulted in significantly better control of blood pressure and lipid profile, and uptake of aspirin, ACE inhibitors and statin therapy. The Alphabet Strategy optimises secondary prevention in subjects with type 2 diabetes mellitus.

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Association between cardiac autonomic neuropathy and hypertension and its potential effects on diabetic complications

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Background and Aim: The association of cardiac autonomic neuropathy (CAN) with hypertension has not been well studied in diabetes. This cross-sectional study aimed to examine this association and its potential role in diabetes complications.

Patients and Methods: We included 310 diabetic patients, 138 type 1 and 172 type 2, 62 of whom were hypertensive and 38 with known coronary artery disease. CAN was assessed on 3 standard tests (deep breathing, lying-to-standing, Valsalva) after withdrawal of drugs able to change heart rate variability, and interpreted according to age.

Results: CAN as defined by at least one abnormal test was found in 123 patients. The prevalence of hypertension was significantly higher in the patients with CAN (31.7% vs 12% ; p<0.0001) and increased from 12% to 23%, 35% and 58% in patients with 0, 1, 2 or 3 abnormal CAN function tests respectively (p<0.0001), in the whole series as well as in type 1 and type 2 diabetic patients taken separately. CAN/hypertension combination was twice more frequent in type 2 than in type 1 diabetic patients (16% vs 8%). The prevalence of this combination increased with age, diabetes duration, and was higher in patients with coronary artery disease, retinopathy, nephropathy, peripheral neuropathy, and postural hypotension (p<0.0001 for all), and this was found also in type 1 and type 2 diabetic patients taken separately. There was no significant association between CAN or hypertension independently with diabetes complications.

Conclusions: The present data strongly suggest that in diabetic patients CAN, defined by defects mainly in cardiac parasympathetic control, may contribute to hypertension, and that the CAN/hypertension combination may be an important contributor to diabetes complications.

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Investigation of cardiac autonomic and peripheral sensory neuropathy in long-standing hypertension with and without type 2 diabetes mellitus

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Background and Aims: The aim of this study was to evaluate cardiac autonomic neuropathy (CAN) and/or peripheral sensory neuropathy (PSN) in long-standing hypertension (HT) with and without type 2 diabetes mellitus (DM). We also tried to find correlations between the duration of HT and DM, the systolic and diastolic blood pressure (BP), the CAN and the PSN.

Materials and Methods: 28 treated hypertensive patients were divided into 2 groups: 18 non-diabetic with normal oral glucose tolerance test (NDHT, age: 54.4±3.3 yrs, HbA1c: 5.74±0.09%, duration of HT: 10.6±2.3 yrs, BP: 134.9/84.2±4.2/2.8 mm Hg, BMI: 28.2±1.1 kg/m^2; mean-SEM), and 10 type 2 DM (DHT, age: 51.0±2.7 yrs, HbA1c: 6.8±0.4%, duration of HT: 12.3±3.1 yrs, duration of DM: 6.1±2.4 yrs, BP: 131.6/85.6±3.9/2.6 mm Hg, BMI: 28.5± 0.9 kg/m2; mean±SEM). 11 healthy controls (C, age: 44.9± 2.2 yrs, BP: 116.8/64.1±3.5/1.9 mm Hg, BMI: 23.7±1.2 kg/m^2; mean±SEM) were also studied. The peripheral sensory

nerve function was characterized via detection of current perception thresholds (CPTs) measured with a Neurometer (Neurotron Inc.) on the median and the peroneal nerves. CAN was assessed by means of five standard cardiovascular reflex tests and a CAN score was calculated (from 0 to 10).

Results: There were no statistical differences between the two HT groups in BMI, duration of HT and BP. The CAN score was close in the two HT groups but they also statistically differed form group C (4.6±0.6 *vs.* 0.46±0.28, 3.9±0.6 *vs.* 0.46±0.28 in order NDHT *vs.* C, DHT *vs.* C; $p<0.001$, mean±SEM,). All the parasympathetic tests and the lying-to-standing test were impaired in both HT groups compared to group C. In the NDHT group the duration of HT positively correlated with the CAN score ($p=0.003$, r=0.66). In both HT groups higher CPT values indicating hypaesthesia were found at the peroneal nerve: in group NDHT at 250 Hz (238.2±67.7 microA; mean±SEM,) and in group DHT at 250 Hz (221.8±88.2 microA; mean±SEM,) and 5 Hz (173.5±92.2 microA, mean±SEM,). Both the duration of HT and DM positively correlated with CPTs measured on the lower extremities at 250 Hz (HT: $p=0.03$, r=0.7; DM: $p=0.02$, r=0.7) and 5 Hz (HT: $p=0.03$, r=0.7; DM: $p=0.008$, r=0.8) in group DHT. Neither the systolic nor the diastolic BP not correlated with the CAN.

Conclusion: Severity of CAN and PSN was similar in NDHT and DHT patients. In the case of HT complicated with DM the PSN can affect more types of neurofibers. Hypertension should be considered as pathogenetic factor for the development of nerve dysfunction. Our data may provide further support, that vascular factors may play an essential role in the development of neuropathy.

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Impairment of peripheral nerve function in obese subjects without diabetes. Influence of hypertension

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Backgrounds and Aims: Impairment in nerve function has been reported in recently-diagnosed type 2 diabetic patients, and some reports also suggest such impairment in non diabetic obese subjects. The aim was to evaluate extensively clinical symptoms, peripheral sensory function and cardiac autonomic neuropathy (CAN) in patients with obesity but without diabetes.

Materials and Methods: We explored 41 obese patients (34 women, 7 men; body mass index 41.1±6.4 kg/m^2) without diabetes according to 75-g oral glucose tolerance test (mean fasting blood glucose 4.73±.63 mM, 2-hr blood glucose 6.60±1.78 mM). Seventeen of them were hypertensive. The symptoms and signs of peripheral neuropathy were graded using Neuropathy Total Symptom Score-6 (NTSS-6) and the Michigan Neuropathy Screening Instrument (MNSI). Current perception threshold for the upper and lower limbs was evaluated using the Neurometer® device at three frequencies (2000, 250, 5 Hz), that assess large and small myelinated and small unmyelinated sensory nerve fiber function (Aβ, Aδ and C, respectively). The Medoc® system (TSA II thermo analyser and WSA3000 vibratory analyser) was used to detect cold, warm and vibration perception thresholds. CAN was defined as at least one abnormal out of 3 heart rate variation standard tests. Sympathetic and parasympathetic activities were investigated using Finapress® device.

Results: NTSS-6 and MNSI identified 2 (4.9%) and 10 (24.4%) patients with clinical neuropathy. According to Neurometer and Medoc tests, C fibers were the most often impaired, with Neurometer 1 or 2 out of 12 parameters recorded were abnormal in 51.3% or 25.6% of the patients, respectively and with Medoc it was in 77.1% or 74.3%. Both 2 Neurometer parameters were abnormal in 23% of the patients. CAN was identified in 45.5%. The predictors for one abnormal Neurometer parameter were hypertension (76.5% vs 31.8%, $p<0.01$), male gender (100% vs 40.6%, $p<0.01$) and low sympathetic activity ($p<0.05$).

Conclusions: Peripheral and autonomic nerve function is often altered in obese people without diabetes. Hypertension might contribute to peripheral nerve dysfunction.

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Higher fasting glucose levels within the normal range are associated with sensory nerve dysfunction in patients with essential hypertension

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Background and Aims: On the one hand, increased cardiovascular risk factors are associated with neuropathy in diabetic patients. On the other hand, autonomic and peripheral sensory neuropathy may be present in patients with essential hypertension, but their prevalence and risk factors has not been identified up to now. We aimed to assess the prevalence and risk factors for autonomic and peripheral sensory nerve dysfunction in patients with essential hypertension.

Materials and Methods: Seventy-two patients with essential hypertension and 31 age-matched healthy control subjects were examined. The five standard cardiovascular reflex tests and heart rate variability (HRV) analysis were performed to define autonomic function. HRV was charac-

terized both by HRV triangular index (HRVTI) and spectral parameters of frequency domain methods. Peripheral sensory nerve function on median and peroneal nerves was characterized by current perception threshold (Neurometer CPT), measured by a neuroselective diagnostic stimulator (Neurotron, Baltimore, MD).
Results: 37.5% of patients had at least one abnormal cardiovascular reflex test, while at least one abnormal HRV parameter was observed in 87% of patients. The prevalence of sensory neuropathy was 15%. Elevation of fasting glucose within the normal limits correlated with higher CPT values indicating hypaesthesia at median nerve testing (2000 Hz: p=0.002; 250 Hz: p=0.03; 5 Hz: p=0.01). Classifying patients into subgroups by fasting glucose (<5.2; 5.2–5.5; 5.6–6.0 mmol/l) higher CPT values were associated with increasing fasting glucose levels even after adjustment for other cardiovascular risk factors (ANCOVA: N. medianus 2000 Hz: p=0.003; 5 Hz: p=0.002). Peripheral sensory nerve dysfunction was also associated with weight, elevated 24-hour mean blood pressure, and triglycerides (all p<0.05). Lower heart rate response to deep breathing (p<0.0001), standing and Valsalva manoeuvre (both p<0.05) was found in patients with essential hypertension compared to control subjects. Compared to healthy controls, hypertensive patients had lower HRVTI (p<0.05), lower total power (p=0.01), and low frequency power (p<0.01). Autonomic dysfunction was independently associated with age (p<0.001), weight (p<0.05), and female gender (p=0.001).
Conclusion: Autonomic and sensory neuropathy are frequent complications among patients with essential hypertension. Age, weight and female sex were independent determinants of autonomic impairment. In these patients peripheral sensory neuropathy was independently associated with higher fasting glucose levels within the normal range, just as with weight, higher 24-hour mean blood pressure and triglycerides. Similarly to observation among diabetic patients, traditional cardiovascular risk factors are associated with sensory nerve dysfunction among patients with essential hypertension as well.

PS 89

Hypertension

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Ambulatory blood pressure monitoring in normotensive and normoalbuminuric type 1 diabetic patients

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Background and Aims: Elevated blood pressure (BP) levels are frequently observed in patients with type 1 diabetes mellitus (DM1). Clinical nephropathy is preceded by microalbuminuria (MAL) which is associated with increased BP and/or abnormalities in circadian rhythm. Ambulatory blood pressure monitoring (ABPM) is a sensitive method to detect early increases in BP in DM1 patients and is a better predictor of vascular events and mortality than casual BP.
The aim of the study was to examine patterns of BP in normotensive and normoalbuminuric DM1 patients during 24 h. using a ABPM (Spacelabs 90207) device.
Materials and Methods: 94 normotensive and normoalbuminuric DM1 patients (age 32.8±7.8 yr., 37 males, 14.8±6.0 yr. of evolution of the disease, BMI 24.7±3.1 kg/m^2, HbA_{1c} 7.6±1.0%, MAL 8.4±6.6 mg/24 h., insulin dose 0.7±0.2 U/kg) were evaluated. Systolic BP (sBP) and diastolic BP (dBP) were recorded for 24 h., daytime and nightime. Night/day BP ratios and percent nightime BP fall were determined. Subjects with a nocturnal fall in either sBP or dBP of less than 10% of daytime values were classified as non-dippers.
Results: Mean±SD ABPM (systolic/diastolic in mmHg) measurements were: 24 h.: 114.5±8.4 and 69.9±6.2; daytime: 116.9±8.9 and 72.6±6.7 and nightime: 106.5±8.7 and 60.9±7.0. Mean night/day sBP and dBP ratios were 1.1±0.1 and 1.2±0.1 mmHg respectively. Mean percent nightime sBP fall and dBP fall were −9±5 and −16±7% respectively. 48 (51%) of the patients were dippers and 46 non-dippers. There were no statistically significant differences in relation to the evolution of diabetes (patients >15 yr. vs. <15 yr.) concerning HbA_{1c}, MAL, BP or percent of non-dippers. Comparing dippers with non-dippers, there were no differences in terms of age, evolution of the disease, BMI, HbA_{1c} or MAL. Patients were also analyzed according to percentiles of MAL: below the 25th percentile (<4.1 mg): 23 patients; 25th–50th percentile (4.1–8.4): 38 patients and above the 50th percentile (>8.4): 29 patients. Patients with MAL below the 25th percentile displayed significantly lower sBP and dBP (p<0.05) than patients with higher percentiles. There were no differences between percentile groups in relation to the other parameters studied.
Conclusion: There is a high prevalence of alterations in the patterns of BP in normotensive and normoalbuminuric DM1 patients. Patients with the lowest percentile of MAL showed significantly lower BP levels.

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Arterial stiffness and microalbuminuria in subjects with type 2 diabetes

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Background and Aims: Microalbuminuria is a risk factor for atherosclerosis in subjects with type 2 diabetes (T2D). Pulse wave velocity (PWV) represents a marker of arterial stiffness and it is increased in T2D. However, little information exists regarding the relationship between microalbuminuria and arterial stiffness in subjects with T2D. In this study we examined the association between PWV and microalbuminuria in diabetic subjects.
Materials and Methods: A total of 84 males with T2D [mean±SD age: 62.3±9.5 years; duration of diabetes 9.7±8.2 years] were studied. All patients underwent clinical and biochemical study. Urinary albumin secretion was assessed by measurement of the 24-hour urine albumin excretion. Arterial stiffness was assessed by measurement of aortic and radial PWV (elastic and muscular artery, respectively). All analyses were preformed using the SPSS statistical package.
Results: Patients with microalbuminuria had higher aortic-PWV values (P=0.02) as well as radial-PWV (P<0.001) in comparison with patients without microalbuminuria. Univariate linear regression analysis showed that aortic-PWV was positively associated with age, duration of diabetes, systolic arterial pressure, pulse pressure, plasma creatinine and urea, history of hypertension and negatively with glomerular filtration rate. Multivariate analysis after adjustment for these associated factors, demonstrated an independent relationship between aortic-PWV and duration of diabetes (b=0.24, P=0.04) and degree of microalbuminuria (b=0.26, P=0.02). Univariate linear regression analysis showed positive associations between radial-PWV and plasma glucose and triglycerides, smoking status and the degree of microalbuminuria. After multivariate adjustment only plasma triglycerides (b=0.20, P=0.06), smoking status (b=0.27, P=0.01) and degree of microalbuminuria (b=0.28, P=0.007) were associated with radial-PWV.
Conclusion: Patients with T2D and microalbuminuria have stiffer arteries than diabetic subjects without microalbuminuria. In addition, microalbuminuria is independently associated with arterial stiffness in both elastic and muscular arteries. Therefore, microalbuminuria may represent an early marker of a widespread arterial dysfunction in diabetic subjects. Interventions aiming at preventing or reducing microalbuminuria might result in improvement of the elastic properties of the arteries and the subsequent high risk for macrovascular complications in subjects with T2D.

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Endothelial function and glucagon-like peptide-1 (GLP-1) in normotensive normoalbuminuric type 2 diabetes subjects with impaired circadian blood pressure variation

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Background and Aims: In individuals with diabetes loss of circadian blood pressure variation (i.e. lack of nocturnal blood pressure dip by at least 10 mmHg, 'non-dipping') is associated with an increased mortality rate. Non-dipping phenomenon is often found in essential hypertension, and in diabetes patients it additionally might be associated with autonomic neuropathy and renal function impairment. Little is known on endothelial function in normotensive diabetes subjects with blunted blood pressure rhythm ('non-dippers'). Recently, glucagon-like peptide-1 (GLP-1), an incretin hormone with therapeutic potential in diabetes, has been suggested to increase blood pressure and heart rate through sympathetic stimulation. However, it has never been studied in non-dipper subjects with diabetes.
Materials and Methods: The study group comprised 27 normotensive normoalbuminuric type 2 diabetes patients (19 women, 13 men, mean [±SD] age 58±7 years, BMI 29.3±2.7 kg/m^2, diabetes duration 7.3±4.7 years, HbA_{1c} 8.1±1.2%) with impaired blood pressure variation, the controls were 18 normotensive normoalbuminuric type 2 diabetes subjects (11 women, 8 men, mean [±SD] age 61±9 years, BMI 30.9±3.4 kg/m^2, diabetes duration 6.8±2.8 years, HbA_{1c} 7.8±0.9%) with normal blood pressure rhythm. HbA_{1c} values in the study group and controls were 7.8±0.8 and 8.0±1.1%, respectively (p>0.05). Fasting serum endothelin-1, sE-selectin, intercellular (ICAM) and vascular cell adhesion molecules (VCAM) as well as tumor necrosis factor-α (TNF-α) and aldosteron were assessed with ELISA method, GLP-1 concentration was measured with immunofluorescence method.
Results: In the study group and controls fasting serum endothelin-1 was 7.5±1.2 and 6.7±0.9 pg/ml (p<0.05), sE-selectin 24.1±10.8 and 17.9±10.5 ng/ml (p<0.05), ICAM 1266±548 and 676±512 ng/ml (p<0.01), VCAM 1792±691 and 1359±548 ng/ml (p<0.05), and GLP-1 5.9±0.9 and 5.1±0.8 pmol/l (p<0.05), respectively. No statistically significant differences were found in serum TNF-α (78.1±91.1 and 74.3±81.9 pg/ml, p>0.05) and aldosteron

(167±36 and 161±27 pg/ml, p>0.05) concentrations between the groups.
Conclusion: Normotensive normoalbuminuric type 2 diabetes patients with impaired blood pressure variation present with marked endothelial dysfunction and elevated fasting GLP-1 levels when compared to the subjects with normal blood pressure rhythm. Further studies are vital to elucidate causal associations between these findings.
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Inverse correlation between serum free IGF-1 and IGFBP-3 levels and blood pressure in type 1 diabetes. A possible explanation of pathogenesis of diabetic complications

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Background: Although in mouse model the gene coding for IGF-1 is considered one of the most important to regulate blood pressure, few discordant data are available in the literature regarding a possible relationship between blood pressure and serum free IGF-1 values in humans. No information is available on type 1 diabetes patients.
Aim: To analyze the relationship between systolic and diastolic blood pressure and serum free IGF-1 and IGFBP-3 levels in subjects in 246 patients with type 1 diabetes without anti-hypertensive therapies. Following the recent guidelines of International Diabetes Federation we decided to use systolic pressure ≤130 mmHg and diastolic pressure ≤80 mmHg for the cut-offs to be considered in this study.
Results: A highly significant inverse correlation has been observed between serum free IGF-1 levels and both systolic (p<0,0001) and diastolic blood pressure (p=0,002) in type 1 diabetes. Similar significant relationships were observed for IGFBP-3 (p=0,005), whose levels were also significantly and directly correlated with those of free IGF-1. This correlation was confirmed by analysis of the free IGF-1 and IGFBP-3 levels after age and gender correction, in subjects with normal or high systolic or diastolic blood pressure. In patients with diabetic rethinopathy (pts=32) IGF-1, IGFBP-3 and their ratio were significantly lower
Conclusion: Lower levels of Free IGF-1 and IGFBP-3 are linked to an increase of blood pressure in type 1 diabetes. This linkage could influence significantly and explain the presence of diabetic complications.

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Soluble CD40 ligand and C-reactive protein concentrations in essential hypertension: effects of hyperglycaemia and hyperinsulinaemia.

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Background and Aims: Subclinical inflammation and platelets activation are involved in the pathophysiology of cardiovascular disease (CVD). Recently both sCD40L (a molecule released by activated platelets) and hs-CRP (an inflammatory marker) have been reported higher among hypertensive patients, but factors accounting for the large variability of both parameters in hypertensive subjects are poorly understood. To measure hs-CRP and sCD40L in 89 CVD event-free men with essential hypertension.
Materials and Methods: We studied 89 patients (mean age 49±11 yrs, range of BMI: 18.4–34.5 kg/m2, serum creatinine below 1.2 mg/dl and cystatin-C concentration ranging from 0.53 to 1.18 mg/l). All subjects underwent a 24 hour ABPM and an OGTT. Lipid and lipoprotein concentrations were measured in a fasting blood sample. Specific immunoassay were used for sCD40L and hs-CRP. Among these subjects, 18% had IFG, 8% IGT, 5% both conditions. Metabolic syndrome (MS, NCEP/ATPIII criteria) was present in 30 out of 89 subjects (34%). Data were analyzed by a SPSS package and were expressed as mean±sd or range.
Results: Mean levels of hs-CRP were 2.4±2.3 (range 0.4 to 8.5) ng/ml; mean levels of sCD40L 8.8±5.0 (range 0.8 to 18.5) ng/ml. No correlation was observed between hs-CRP and sCD40L (p=0.97) and no differences in both parameters emerged in subjects with and without MS. Neither hs-CRP and sCD40L did correlate with age, BMI, systolic and diastolic 24 h ABPM parameters, renal function indexes or urinary albumin excretion. Within lipid parameters, a weak positive regression was observed between total cholesterol and sCD40L (p=0.047). hs-CRP strongly correlated with fibrinogen levels (p<0.0001). sCD40L positively correlated with fasting glucose (r=0.23, p=0.029), basal insulin levels (r=0.34, p=0.002), and HOMA-IR (r=0.37, p=0.001), while hs-CRP weakly correlated with the insulinogenic index (r=0.25, p=0.05) and the area under the OGTT curve for insulin (r=0.32, p=0.027). In a multiple regression model, HOMA-IR emerged as the main determinant of the variability of sCD40L levels, while AUC insulin was the main determinant of the variability of hs-CRP concentrations.

Conclusion: Minor changes in glucose and insulin metabolism might account at least in part for the inflammatory disorders related to essential hypertension.

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Exogenous insulin and hypertension in patients with type 2 diabetes

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Background and Aims: The influence of exogenous insulin and consequent peripheral hyperinsulinemia on blood pressure are still controversial.Aim of this study was to investigate the relations between insulin therapy and hypertension in patients with type 2 diabetes.
Materials and Methods: We studied 466 consecutive type 2 diabetics(236 M,230 F,age 64.9+−8.8 yrs).Presence of hypertension(H)(defined as blood pressure>140/90 mmHg or taking hypotensive therapy),glycemic control(in terms of fasting glycemia and HbA1c over the last 10 years), duration of diabetes,body mass index(BMI),and the presence of microvascular(nephropathy and retinopathy) and macrovascular(coronary heart disease and peripheral vascular disease) complications were recorded.According to the type of therapy,patients were divided in insulin treated (IT)(treated with insulin alone or associated with oral agents) and non insulin treated(NIT)(treated with oral agents or only diet).
Results: 88 patients(18.8%) were IT,being the remaining fraction (81.2%) NIT.The prevalence of H was similar in the 2 groups (78.4% vs 82.8%,p=NS).Systolic blood pressure was similar in IT and NIT,whereas diastolic blood pressure was lower in the former than in the latter(91.3+−10.2 vs 93.9+−10.8 mmHg,p=0.04). No correlation was found between insulin daily dosage and systolic or diastolic blood pressure (respectively r=−0.07 and r=0.13). Insulin daily dosage was lower in hypertensives than in normotensives (31.2+−18.5 vs 45.7+−29 U/die,p=0.01). H frequency was similar in IT and NIT in normoweight (BMI<25),overweight(BMI 25− 29.9) and obese (BMI> 30) patients. Stratifying subjects by fasting glycemia (<140; 141−180;>180 mg/dl) and HbA1c(<7;7–8;>8%) H frequency was not significantly different in any stratum in IT and NIT;the same result was observed stratifying subjects by duration of diabetes(<5;6–10;>10 yrs).H frequency was similar in IT and NIT in patients with normo-(<30 mg/24 h), micro(30–299 mg/24 h) or macroalbuminuria (>=300 mg/24 h),as well as in those with or withouth diabetic retinopathy(background and proliferative).Similarly,the proportion of hypertensive and normotensive subjects was not significantly different in relation to the presence or not of coronary heart disease or peripheral vascular disease in IT and NIT.
Conclusion:The results of this study show no relation between insulin therapy and hypertension,and therefore they are not supportative of the hypothesis that exogenous insulin may increase blood pressure in type 2 diabetics.

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Candesartan improves insulin sensitivity as compared to standard treatment with hydrochlorothiazide in hypertensive subjects with abdominal obesity - the MEDICA study

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Background and Aim: Several studies have indicated that blockers of the type 1 angiotensin II receptor (ARBs), e.g. candesartan, are favorable in comparison to standard antihypertensive therapy with beta blockers and thiazide diuretics with respect to metabolic effects. In particular, this has been shown for the risk for development of type 2 diabetes. The MEDICA study was performed to identify underlying *me*chanisms for the *di*abetes-preventing effect of *ca*ndesartan. The primary objective was to compare insulin sensitivity during candesartan (C) and hydrochlorothiazide (H) treatment. Comparisons were also made with placebo (P).
Patients and Methods: 26 abdominally obese (waist >102 cm M, >88 cm F), non-diabetic, hypertensive patients were randomized to 12-wk treatment periods, in a 3-way crossover-design, with C (16–32 mg), H (25–50 mg) and P, respectively. Mean age was 54.8±10.4 (SD) yr and BMI 30.2±3.0 kg/m^2. A euglycaemic hyperinsulinaemic (56 mU/m^2/min) clamp and other assessments were performed at the end of each treatment period. 22 subjects (10 M, 12 F) completed the study and were available for analysis.
Results: Insulin sensitivity following C was higher than following H (p=0.004) but not significantly different from P, and M-values were: C 6.63±2.04, H 6.07±2.05 and P 6.90±2.10 mg/kg BW/min. HbA1c was higher (p=0.035) following H than P (C 4.5±0.4, H 4.6±0.6, P 4.3±0.4%). There were no statistically significant differences between treatments in body weight, serum total, HDL and LDL cholesterol, triglycerides or in 1st phase insulin secretion (AUC for insulin during the first 10 min of an IVGTT). Systolic and diastolic blood pressures were reduced by C

and H compared to P (135±15/88±9, 138±8/91±7 vs 150±9/96±7 mmHg, respectively, p<0.01). In addition, DBP was significantly lower for C than H (p=0.009). Additional analyses in this study will address other mechanisms, e.g. vascular and autonomic nerve regulation and adipose tissue distribution and function, that may have an impact on glucose and lipid metabolism.

Conclusions: Insulin sensitivity was higher following candesartan than hydrochlorothiazide treatment. This may partly explain previously demonstrated favorable metabolic outcomes with candesartan and other ARBs as compared to antihypertensive regimens that include thiazide diuretics.

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Elevated blood pressure during exercise is related to insulin resistance and central obesity in offspring of type 2 diabetic patients

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Background and Aims: We hypothesize that insulin resistance and related metabolic disorders are associated with augmented sympathetic activation which results in elevation of blood pressure and heart rate during physical exercise. Thus, we studied cardiovascular responses to exercise in offspring of type 2 diabetic patients and in control subjects.

Materials and Methods: According to the distribution of insulin sensitivity assessed by the hyperinsulinemic, euglycemic clamp in a population of 119 healthy offspring of type 2 diabetic patient, we selected 1st and 4th quartiles to represent insulin resistant (IR, n=30) and insulin sensitive (IS, n=31) groups. Control group (n=19) consisted of healthy subjects without family history of type 2 diabetes. Blood pressure and heart rate were measured at rest (1 MET=VO_2 of 3.5 ml*kg^{-1}*min^{-1}), during exercise at intensities corresponding to 3 MET, 5 MET, 7 MET, 9 MET and recovery phase of the cardiopulmonary exercise test. Other main examinations were anthropometric measurements, and an oral glucose tolerance test to exclude diabetes. Furthermore, abdominal fat distribution was evaluated by computed tomography.

Results: Blood pressure and heart rate were comparable between study groups at rest. Compared to the control group the IR-group had higher systolic and diastolic blood pressure at 5 MET and 9vMET exercise levels and during recovery (P<0.05 for all). In addition, the IR-group had higher heart rate at 5 MET level as compared with that in the IS-group (P<0.05) and in the control group (P<0.01). The only difference between the IS-group and the control group was higher diastolic blood pressure in the IS-group at 9 MET level (P<0.05). Other factors associated with exercise blood pressure response were body mass index and waist circumference for systolic blood pressure (P<0.01 for both) and intra-abdominal fat area and waist circumference for diastolic blood pressure (P<0.01 and P<0.05, respectively). After adjustment for these confounding factors the differences between the groups disappeared with respect to hemodynamic responses to exercise.

Conclusion: Our results suggest that in insulin resistant offspring of type 2 diabetic patients the cardiovascular system becomes strained due to the hyperdynamic state which is induced by physical exercise already at moderate intensity corresponding to some daily activities. The higher pressor response to physical activity in offspring of type 2 diabetic patients with insulin resistance may be attributed to extra load caused by overweight and central obesity.

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Effectiveness of nurse led hypertension clinic in Wolverhampton Diabetes Centre - a report of 4-year clinical experience

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Background and Aims: An effective blood pressure (BP) control remains difficult to achieve in patients with Diabetes Mellitus (DM) despite its undisputed benefits. Possible explanations include poor patient education, non-compliance, the need for multiple antihypertensive agents, and failure to titrate their dose within a reasonable time period. A nurse led, protocol driven hypertension clinic was established in Wolverhampton Diabetes Centre to address these issues. We report the results of a retrospective audit undertaken to assess the implementation of the protocol and its effectiveness in optimising blood pressure control.

Material and Methods: Patients with established complications of DM with suboptimal BP control despite antihypertensive treatment, were referred to this clinic. The referral form included the intended sequence of antihypertensive therapy according to a protocol which is based on the compelling indications and contraindications for clinical

characteristics of the individual. During the clinic visits BP was measured in a standardised manner and antihypertensive therapy was initiated or titrated according to the protocol. Adverse events were reported to the referring clinicians. Patients were discharged when the intended sequence of therapy was completed or target BP was achieved.

Results: 131 patients were referred to the clinic between August 2001 and September 2005. 2 patients defaulted, 1 died and 6 were considered inappropriate referrals. The majority had type 2 DM, 94% were insulin treated, 57% patients were obese, 50% had retinopathy and 76% had proteinuria. There was a significant reduction in systolic (186±24 to 139±12 mm Hg, mean±SD $p<0.05$) and diastolic (85±13 to 67±11 mm Hg) BP after a mean of 3.3 clinic visits (range 2–13). 64% achieved systolic BP<140 mm Hg at the time of discharge. 51 (62%) of the 83 patients who had ambulatory BP monitoring prior to discharge had a mean systolic BP of<140 mm Hg. The total number of antihypertensive agents used increased from 2.5±1.2 to 3.1±1.1 and the number for which the dose was maximised increased from 1.2±1.1 to 2.1±1.2. In 65 (49%) patients number of antihypertensive agents used remained the same or were reduced. Improvements in total cholesterol (4.8+/−1.3 to 4.3+/−0.9) and glycaemic control (8.4+/−1.8 to 8.0+/−1.7) were also noticed.

Conclusions: A systematic approach in a nurse led hypertension clinic led to an effective BP control in a significant proportion of patients. In approximately half the patients, same or fewer numbers of tablets were used due to a more effective dose titration. A more favourable biochemical profile may have been a result of opportunistic interventions and education.

1044

Blood pressure control in diabetic patients in the eight newly accessed EU countries

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Background and Aims: To determine blood pressure (BP) control in diabetic patients in the Czech Republic, Estonia, Hungary, Lithuania, Latvia, Poland, Slovakia and Slovenia.

Materials and Methods: 11.084 patients (type 1, n=2.497, type 2, n=8.231, other types, n=356) were randomly enrolled. Data on BP values, diagnosed arterial hypertension, use of antihypertensive drugs, and chronic diabetes complications were collected in a survey by diabetologist or endocrinologist at regular visits to diabetes centres.

Results: Only 8.6% of type 2 (T2), and 41.9% of type 1 (T1) patients had BP <130/80 mmHg. Hypertension was diagnosed in 31.1% of T1 and 81.0% of T2 patients. Percentages of drug-treated hypertensive patients under/over the following combined SBP and DBP cut-off values were:

BP mmHg	All	T1	T2
n	7488	762	6543
<130/80	5.9	11.8	5.2
<140/90	30.5	40.9	29.3
<160/90	57.0	64.9	56.1
≥160/90	13.5	9.4	13.9

The respective results in patients with micro- and macrovascular complications were:

BP mmHg	Nephropathy			Retinopathy		
	All	T1	T2	All	T1	T2
n	2837	694	2074	3626	992	2547
<130/80	11.3	28.5	5.6	11.4	26.5	5.6
<140/90	41.9	66.5	33.6	42.3	65.2	33.3
<160/90	65.5	81.1	60.4	65.0	81.5	58.5
≥160/90	10.3	4.4	12.2	11.8	5.5	14.2

Stroke/TIA			Coron. art. disease			Periph. art. disease		
All	T1	T2	All	T1	T2	All	T1	T2
639	27	595	3550	198	3285	1470	150	1282
6.3	14.8	5.9	8.0	13.4	7.0	8.0	13.0	7.4
31.4	33.3	31.1	35.5	42.2	34.9	34.7	49.3	32.6
58.3	66.6	57.7	60.4	68.0	60.0	62.2	76.7	60.4
14.2	18.5	14.2	12.8	12.8	12.8	11.8	8.2	12.3

Conclusion: The level of BP control is highly unfavourable in diabetic patients attending diabetes centres in the eight newly accessed EU countries. Only a small proportion of diabetics treated for hypertension and those with chronic complications requiring strict BP control eventually achieve BP target values, and a considerable proportion of them are in a very poor BP control. The results from the survey call

for a much more intensive BP lowering to achieve target values.
Supported by Novo Nordisk

PS 90

Nephropathy - clinical interventions

1045

Effect of the oral PKC β *post hoc* analysis of renal safety data from two diabetic retinopathy trials

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Background and Aims: Ruboxistaurin (RBX), an oral PKC β inhibitor, reduced visual loss in two long-term phase 3 diabetic retinopathy studies. A one-year diabetic nephropathy pilot trial demonstrated that RBX improved albuminuria and was not associated with significant decline in estimated glomerular filtration rate (eGFR). This *post hoc* analysis of the PKC-DRS and PKC-DRS2 diabetic retinopathy trials (n=813) evaluates the long-term impact of RBX on renal outcomes.
Materials and Methods: We performed a *post hoc* analysis of combined data from two three-year, randomized, double-blind, parallel, multi-center, placebo-controlled, phase 3 studies of RBX 32 mg/day. Patients (placebo: n=401, RBX 32 mg/d: n=412) had moderate to severe (AAO severity scale; equivalent to ETDRS score ≥47A and ≤53E) nonproliferative diabetic retinopathy and well-preserved baseline renal function. Renal outcomes were defined as: 1) doubling of serum creatinine; 2) progression to advanced (stage 4: eGFR ≤30 mL/min/1.73 m^2) chronic kidney disease (CKD) from an earlier stage (Note: Patients in the PKC-DRS2 study had few ESRD events. Thus, low GFR was used instead of ESRD as a renal outcome); or 3) death. The Modification of Diet in Renal Disease formula was used to calculate eGFR.
Results: At baseline, the mean eGFR (±SD) was 83.3± 27.9 mL/min/1.73 m^2 and 20.7% of patients had CKD (eGFR ≤60 mL/min/1.73 m^2). Renal outcomes in the RBX-treated group did not differ from those receiving placebo. During the trials, 6.8% of patients experienced doubling of serum creatinine (RBX: 6.1%, placebo: 7.5%, p=NS), 5.0% progressed to advanced CKD (RBX: 4.9%, placebo: 5.0%, p=NS), and 5.0% died (RBX: 4.4%, placebo: 5.7%, p=NS). At least one renal outcome occurred in 13.2% of patients (RBX: 12.1%, placebo: 14.2%, p=NS). From baseline to endpoint of the three-year studies, eGFR decreased by 10.6±20.7 mL/min/1.73 m^2 (RBX: −10.1±20.7, placebo: −11.1±20.8, p=NS). Decline in eGFR was related to five baseline variables: diabetes duration, diabetes type, alcohol use, blood pressure, and eGFR.
Conclusion: *Post hoc* analysis of long-term trial data demonstrates that 32 mg/d RBX has no negative impact on renal outcomes or eGFR in diabetic retinopathy patients with well-preserved baseline renal function. The effect of RBX on renal outcomes must be confirmed using large-scale prospective trials powered to evaluate the efficacy of RBX in this population.
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Antiproteinuric effects of telmisartan versus valsartan in patients with type 2 diabetes and overt nephropathy

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Background and Aims: Proteinuria is a key indicator of renal and cardiovascular health. Reducing proteinuria is a central aim to prevent progression in patients with type 2 diabetes mellitus (T2D) and nephropathy. Telmisartan is an angiotensin receptor blocker (ARB) with 24-h plasma half-life that has demonstrated efficacy in slowing glomerular filtration rate decline in T2D. This study (VIVALDI) directly compared the efficacy of telmisartan with that of valsartan in this patient population.
Materials and Methods: This 1-year, prospective, double-blind, parallel-group study randomized 885 patients with T2D, hypertension (seated SBP/DBP >130/80 mmHg), macroalbuminuria (≥900 mg/24 h) and a serum creatinine of 97–265 μmol/L in women and 115–265 μmol/L in men to either telmisartan 80 mg/day or valsartan 160 mg/day. To ensure blood pressure control (<130/80 mmHg), α- or β-blockers, calcium channel blockers, or diuretics were allowed. The primary analysis was a non-inferiority test of 24-h urinary protein excretion rate (UPER).
Results: In the full analysis set, geometric mean UPER (95% CI) was reduced by 33% (27%, 39%) of baseline with telmisartan (n=431) and by 33% (27%, 38%) with valsartan (n=431), demonstrating non-inferiority. Results were similar for the per-protocol analysis. No significant differences were seen in changes in serum creatinine, creatinine clearance or estimated glomerular filtration rate. In the two groups, a trend towards greater efficacy was seen among patients with better blood pressure and diabetes control, and

in patients who had not previously received ARBs or ACE inhibitors, indicating the importance of these modalities for improving outcomes in this patient population.
Conclusion: In patients with T2D and proteinuria, telmisartan provides similar reductions in proteinuria to valsartan. Treatment with telmisartan is an effective therapeutic option in these patients.
Supported by Boehringer Ingelheim GmbH

1047

Low-dose aspirin does not affect of antiproteinuric effect of enalapril in microalbuminuric type 2 diabetic patients. A randomised, double-blind, crossover study

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Background and Aims: An interaction between aspirin and angiotensin-converting-enzyme (ACE) inhibitors is pharmacologically plausible through their opposite effects on vasodilators prostaglandins. The aim of this study was to analyze the effect of aspirin on urinary albumin excretion (UAE) and glomerular filtration rate (GFR) of microalbuminuric type 2 diabetic patients treated with the ACE inhibitor enalapril.
Materials and Methods: In this randomized, double-blind, crossover, placebo-controlled study, 18 microalbuminuric (UAE=30–300 mg/24 h) type 2 diabetic patients received aspirin (300 mg/d) or identical placebo for 8 weeks, with a 6-week washout period. The patients were aged 56±9 years, with a diabetes duration of 16±7.5 years; 11 (61%) were female, and were all using enalapril 10 mg bid. GFR was measured by ^{51}Cr-EDTA single-injection method, UAE by imunoturbidimetry and expressed as median (range). Sample-size calculation disclosed a minimum of 17 patients to detect a 30% change in UAE, greater than the mean coefficient of variation of UAE (alfa 0.05 and beta 0.20).
Results: There were no significant statistical differences at baseline between placebo or aspirin groups, regarding GFR (113±35 vs. 112±34 ml/min/1.73 m^2; P=0.91) and UAE [58.6 (6.8–216) vs. 64 (8.2–268) mg/24 h; P=0.66], respectively. After 8 weeks of treatment there were no significant differences between the period using placebo or aspirin, respectively, regarding GFR (108±34 vs. 111±47 ml/min/1.73 m^2; P=0.90) and UAE [57.7 (8.9–420.0) vs. 63 (8.2–272.0) mg/24 h; P=0.45] (Fig. 1). There were also no significant differences between blood pressure (133±16/79±7 vs. 134±11/77±7 mmHg; P=0.41), capillary glycemia (127±46 vs. 131±39 mg/dl; P=0.67) and A1c test (8.0±1.7 vs. 8.6±1.7%), respectively, after placebo and aspirin. There were no period (P=0.41) or carry-over effects (P=0.49).
Conclusion: Low-dose aspirin (300 mg/d) does not interfere with UAE levels of microalbuminuric type 2 DM while concomitantly using an ACE inhibitor.

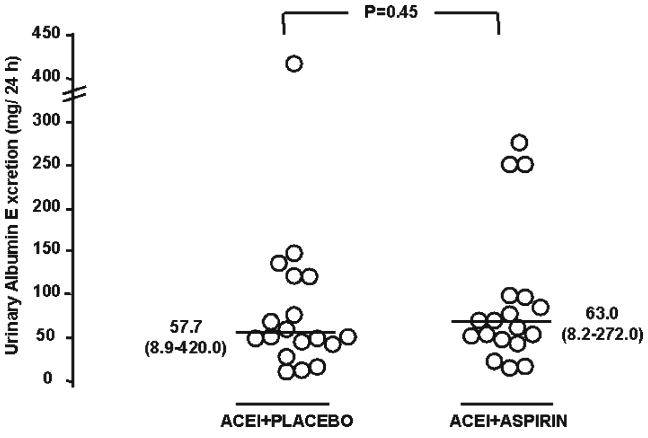

Figure 1 – Urinary albumin excretion (UAE) values after 8 weeks of angiotensin-converting-enzyme inhibitor (ACEI) plus placebo or aspirin

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Irbesartan treatment reduces biomarkers of inflammatory activity in patients with type 2 diabetes and microalbuminuria: an IRMA 2 substudy

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Background and Aims: To evaluate the impact of irbesartan treatment on biomarkers of low-grade inflammation, endothelial dysfunction, growth factors and advanced glycation end products (AGE peptides) during the Irbesartan in Patients with Type 2 diabetes and Microalbuminuria (IRMA 2) study. The study was a two-year multicenter, randomised, double-blind trial in patients with type 2 diabetes mellitus and microalbuminuria comparing irbesartan (150 or 300 mg once daily) versus placebo. Primary endpoint was onset of overt nephropathy.
Materials and Methods: A subgroup (n=269, 68%) was analysed for biomarkers at baseline and after one and two years. We assessed high sensitivity CRP (hs-CRP), interleukin 6 (IL-6), fibrinogen, soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble inter cellular adhesion

molecule-1 (sICAM-1), sE-selectin, transforming growth factor-beta (TGF-beta) and AGE-peptides.
Results: Treatment with irbesartan yielded significant changes in hs-CRP (based on GEE regression coefficient) −24.9 (−35.6 to −12.3)% (95%CI) during follow-up with a 5.4% decrease per year versus the 10% increase per year in the placebo group (p<0.001)), fibrinogen (change, −0.16 (−0.30 to −0.018) g/L with treatment with a −0.059 g/L change per year from baseline versus placebo 0.059 g/L increase per year (p=0.027), and IL-6 (treatment effect, −13.6% (−22.0 to −4.3) with 1.8% increase per year compared to placebo 6.5% increase per year (p=0.005)). Changes in IL-6 were associated with changes in albumin excretion (p=0.04). There was no significant treatment effect on the other biomarkers.
Conclusion: 300 mg irbesartan once daily reduces low-grade inflammation in this high-risk population and this may reduce the risk of cardiovascular disease.

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Impact of carvedilol on microalbuminuria is independent of the statin effect

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Introduction: Both carvedilol and statins by their anti-inflammatory effects improve endothelial function and microalbuminuria. To assess if carvedilol's effect was independent of the statin effect we preformed this study.
Methods: The GEMINI study compared the beta-blocker metoprolol with the combined alfa-beta-blocker carvedilol in a randomized, double-blind, parallel-group, multi-center US trial with 1210 hypertensive type 2 diabetic patients. Microalbuminuria was measured in all patients by radioimmunoassay.
Results: In 388 subjects on carvedilol microalbumin was decreased by 14.0%, and in 542 subjects on metoprolol non-significantly increased by 2.5% resulting in a 16% relative reduction (95% CI 6, 2.5 p=0.003). Over the course of the trial 6.4% of subjects on carvedilol and 10.6% of subjects on metoprolol developed microalbuminuria (OR 0.53, 95% CI 0.30, 0.93 p=0.03). At baseline 183 of the carvedilol group and 247 of the metoprolol group were taking statins. In the carvedilol group there was a 16.8% reduction (p=0.01) and in the metoprolol group a 6.8% increase (p=0.32) in microalbuminuria. Overall there was a 22.2% treatment difference (95% CI 34.8–7.2 p=0.005).
Conclusion: The effect of carvedilol on microalbuminuria was not ameliorated by concurrent statin use. Therefore, it is likely that separate anti-inflammatory pathways are stimulated by statins and carvedilol resulting in improved endothelial function.
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Combined antihypertensive drugs and antihypertensive/statin combination seem to be superior in reducing blood pressure and inflammation

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Background and Aims: Hypertension and inflammation facilitate the progression of nephropathy, particularly when albuminuria is present. The aim of the study was to compare the effect of renin-angiotensin system (RAS) inhibitors and statins alone and in combination on blood pressure, albuminuria, and C-reactive protein (CRP) and fibrinogen (FIB) as pro-inflammatory markers.
Materials and Methods: A total of 328 hypertensive type 2 diabetics (56.48±10.9 yrs. old; diabetes duration 11.2±6.7 yrs.) were studied over a 1-yr follow-up period. Patients were randomized to receive either angiotensin-converting enzyme (ACE) inhibitor lisinopril (group I, n=65), angiotensin receptor blocker (ARB) losartan (group II, n=52) and atorvastatin (group III, n=64), or lisinopril/losartan (group IV, n=45) and atorvastatin/lisinopril combination (group V, n=58). The control group included 44 patients. CRP, FIB, atherogenic index of plasma (AIP), systolic (SBP), diastolic (DBP) and pulse pressure (PP) and albumin excretion rate (AER) were determined at the beginning and at the end of the study. The patients were assigned to groups based on AER/mg/24 h/(<15, 15–30, 30–300, >300) and PP (<45, 45–50, 50–65, >65). Wilcoxon signed ranks test and Kruskall-Wallis test were used in the statistical analysis.
Results: Initial SBP, DBP, PP and AER were significantly reduced in the groups treated with RAS-inhibitors (generally p<0.01), but PP also fell in the group treated with atorvastatin (p=0.009). CRP decreased significantly in all groups (p<0.01), with the exception of Group II (p=0.232), whereas FIB was significantly reduced in the groups I, III and V (p=0.040, p=0.001 and p=0.023, respectively). AIP was significantly reduced in the atorvastatin-treated group (p<0.001). Among-group comparison revealed that the reduction in PP and SBP was most pronounced in Group

IV (both p<0.001), while a difference in DBP reduction was not observed (p=0.147). Group III, followed by Group I, was the most effective in AER reduction. Group I and III did not differ in their effect on CRP and FIB (p=0.134 and p=0.256, respectively), whereas the reduction of CRP and FIB was more pronounced in Groups III and V, mainly in the group treated with the atorvastatin/lisinopril combination (p<0.01). Significant differences were found in initial CRP, FIB and AIP (p=0.01, p=0.004 and p=0.041, respectively) according to AER, and in FIB and AIP (both p<0.001) according to PP.
Conclusion: Blood pressure, AER, AIP and inflammatory markers are among risk factors for the development of nephropathy. ACE-inhibitor/ARB and ACE-inhibitor/atorvastatin combinations seem to be more efficient than these drugs used separately in the reduction of BP and AER, and inflammatory markers, respectively.

PS 91

Nephropathy - clinical

1051

Microalbuminuria is common in Japanese type 2 diabetes: a nationwide survey from Japan Diabetes Clinical Data Management Study Group

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Background and Aims: To elucidate the prevalence of microalbuminuria and clinical nephropathy in a nationwide survey in patients with T2DM.
Materials and Methods: We performed a cross-sectional, multi-center study which consists of 22 medical clinics (from different areas in Japan) using the same software to incorporate patients' record. All consecutive patients who visited from Jan. 2004 to July 2005 and whose diabetes was diagnosed before 2003 were included.Main Outcome and Measurements were urinary albumin to creatinine ratio (ACR, mg/gcr) and serum concentration of creatinine (Scr, mg/dl). Nephropathy stage was defined as follows: I, normoalbuminuria (ACR<30); II, microalbuminuria (ACR >=30 and<300); III, overt nephropathy (ACR >300 and Scr<2.0); IV, renal failure (Scr >=2.0); V, end-stage renal failure (treated with dialysis or renal transplantation).
Results: A total of 8897 patients were included. More than 30% of patients were microalbuminuric, and 10% were suffering from clinical nephropathy and/or renal failure. In patients with normo- or microalbuminuria, ACR was significantly higher in female than in male, and muliple regression analysis revealed that significant determinants of ACR were SBP, HbA1c, age, femaleness, BMI, duration, and triglyceride (F-value 53.9, p<0.0001).
Conclusion: In a nationwide survey of patients with T2DM in Japan, microalbuminuria is common. The result may account for the increasing incidence of end-stage renal failure in Japan.

Clinical characteristics of the subjects

Stage	I	II	III	IV	V
N(%)	5152(58)	2812(32)	657(7)	242(2.6)	34(0.4)
Male%	66	63	72	70	82
BMI	24.1±3.7	24.7±4.0	25.1±4.1	23.8±3.9	23.9±4.0
HbA1c (%)	6.9±1.1	7.3±1.3	7.4±1.3	6.7±1.2	6.5±2.5
Diet/Tablet/Insulin%	23/59/18	15/59/26	9/49/42	14/36/50	15/12/73
SBP (mmHg)	127±15	133±16	137±19	139±22	139±16
DBP (mmHg)	75±10	76±11	76±12	74±12	75±10
LDL-chol(mg/dl)	122±58	121±52	133±82	121±66	103±49
HDL-chol (mg/dl)	55±15	54±15	50±14	47±14	53±20

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Course of microalbuminuria in type 2 diabetic patients: a 5-year follow-up study

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Background and Aims: The aim of this study was to analyze the clinical course of microalbuminuria in a cohort of type 2 diabetes mellitus (DM) patients, evaluating risk factors related to progression to macroalbuminuria and mortality.
Materials and Methods: In this prospective study, 83 microalbuminuric (urinary albumin excretion [UAE] 20–199 μg/min) type 2 DM patients, 51 men (61%), 70 (84%) white and 13 (16%) mixed or black, aged 61±8 years, with diabetes duration of 13±7 years, and median UAE of 47.2 μg/min (range 20–193), were followed for a mean period of 5.2±2.5 years. UAE was measured by immunoturbidimetry, and glomerular filtration rate (GFR) was estimated by the four-component Modification of Diet in Renal Disease equation, at baseline and at follow-up.
Results: Six patients (7%) were lost to follow-up. Three (4%) died from coronary artery disease before renal status re-evaluation. Among the 74 (89%) re-examined patients, 18 (24.3%) progressed to macroalbuminuria (MA), and 6 of them developed kidney failure, 41 (55.4%) remained microalbuminuric (MI), and 15 (20.3%) regressed to normoalbuminuria (NO). There were no differences among the groups concerning the number of patients receiving

angiotensin-converting-enzyme inhibitors at baseline (P=0.31) or at follow-up (P=0.10).Cox proportional hazards analysis (Hazard Ratio [95% Confidence Interval]) showed that baseline UAE above the median, ≥ 47.2 μg/min, (HR: 3.5 [1.05–11.65], *P*=0.042) was related to the progression to macroalbuminuria, higher baseline GFR (0.96 [0.94–0.99], *P*=0.002) was a protector factor for progression, while age (*P*=0.20), and diabetic retinopathy (*P*=0.31) were not significant. Seventeen (23%) patients developed GFR values<60 ml/min/1.73 m^2 at follow-up (11 in MA, 5 in MI, and 1 in NO group [*P*=0.0001]). A Cox proportional hazards analysis disclosed that higher baseline GFR levels (0.95 [0.93–0.97], *P*=0.001) protected against the development of GFR values<60 ml/min/1.73 m^2 at follow-up, while UAE ≥ 47.2 μg/min (*P*=0.20), and age (*P*=0.27) were not significant in the model. The mortality rates were not different among the patients who progressed or not (log rank, *P*=0.46).

Conclusion: In microalbuminuric type 2 DM patients, the progression to later stages of kidney disease was observed in only 25% of the patients and was mainly related to a greater involvement of renal function, expressed by higher baseline UAE levels, and lower baseline GFRs, which also predicted the development of GFR values<60 ml/min/1.73 m^2 at follow-up.

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Long-term prevention of diabetic nephropathy: an audit

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Background and Aims: A risk for developing diabetic nephropathy of 3–30%/year has been reported in type 1 diabetic patients with microalbuminuria not receiving antihypertensive treatment. In 1995 international guidelines recommended angiotensin converting enzyme (ACE) inhibitor treatment in patients with microalbuminuria, as randomised studies demonstrated a risk reduction of 62% for development of diabetic nephropathy in patients treated with ACE inhibitors. The aim of the present 11-year observational follow-up study was to audit the effect of RAS-blocking treatment on progression of microalbuminuria and development of diabetic nephropathy.

Materials and Methods: In 1995 ACE inhibitors were recommended for treatment of microalbuminuric type 1 diabetic patients in our outpatient clinic at Steno Diabetes Center. For audit purpose we consecutively identified all type 1 diabetic patients with persistent microalbuminuria (30–300 mg/24 hours) (n=227). During follow-up, patients visited our outpatient clinic 2–4 times/year, where albuminuria, BP and HbA1c was measured. Development of diabetic nephropathy was defined as urinary albumin excretion rate >300 mg/24 h in 2 of 3 urine samples. Patients were followed until December 31, 2005, death, or emigration.

Results: At end of follow up, a total of 134 patients were still followed at Steno Diabetes Center, 58 patients (26%) had died, and 35 patients (15%) had moved to another part of the country. Mean follow-up time was 8.5 (3.5) years. Throughout the study 78% were treated with a RAS-blocking agent (ACE inhibitor or angiotensin II receptor blocker). In 2005 29 patients were not receiving RAS-blocking treatment, of whom 19 had spontaneously regressed to normoalbuminuria, all of the remaining patients had been recommended RAS-blocking treatment, but did not comply with the treatment, mostly because of adverse effects. Overall there was a mean decline in UAER of 4%/year. During follow-up 60 patients (26%) progressed to overt diabetic nephropathy, corresponding to 3%/year. Of these patients 26 regressed to microalbuminuria and 5 to normoalbuminuria on RAS blocking agents. Median (range) number of antihypertensive agents prescribed was 2 (0–5), 60% of patients with elevated urinary albumin excretion rate received low-dose aspirin and 63% received statins. Mean (SE) systolic BP was 141 (1) mm Hg during follow-up as compared to 142 (1) at baseline (NS). In contrast, diastolic BP was reduced from 80 (1) mm Hg to 77 (1), *P*<0.001. There was a small decrease in HbA1c of 0.2 (95% CI: 0.1 to 0.3)%-points from 8.8% at baseline, *P*<0.01.

Conclusion: In an outpatient clinic, the implementation of RAS-blocking treatment in type 1 diabetic patients with microalbuminuria successfully reduced long-term progression to overt diabetic nephropathy comparably to what has previously been reported in intervention trials of shorter duration.

Supported by the Clinical Development Foundation at Steno Diabetes Center

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Is the decrement in renal function in older compared to younger patients with type 2 diabetes independent of albuminuria status?

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Background and Aims: Diabetic nephropathy is the leading cause of renal failure in the developed world. We aimed to characterize the association between age, albuminuria status, blood pressure and renal function in a cohort of subjects with Type 2 diabetes.

Materials and Methods: In a cross sectional study, glomerular filtration rate (GFR) was measured in 656 unselected subjects with Type 2 diabetes, (age 28–86 years) attending a tertiary referral centre and 49 non-diabetic healthy subjects (potential kidney donors, age 31–65 years) using plasma disappearance of ^{99m}Tc-DTPA.

Results: For subjects aged >55 years (Figure) mean GFR was similar in those with normo-and microalbuminuria, but reduced in those with macroalbuminuria ($p<0.01$). For subjects aged<55 years, GFR was similar regardless of albuminuria status.

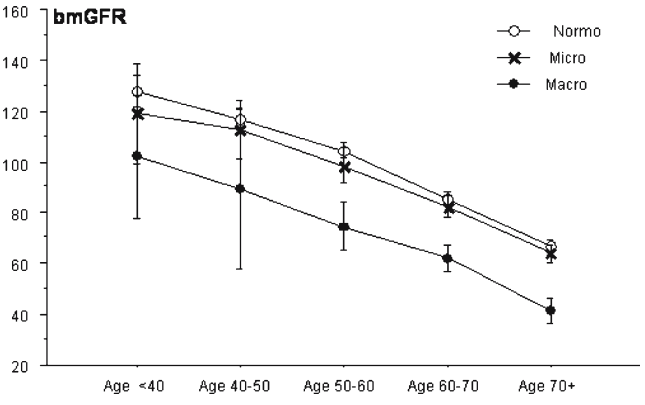
Across different age groups, the decrement in GFR in the non-diabetic subjects was 1.03 ml/min/1.73 m^2/year, consistent with the age related decline of GFR reported in previous studies. In subjects with diabetes, a greater decrement in GFR was observed beyond that expected with ageing alone in all of the albuminuria categories (1.8, 1.6 and 1.8 ml/min/1.73 m^2/year in the normo-, micro- and macroalbuminuric groups respectively).

Decrements in GFR with age in subjects on antihypertensive therapy (AHT) were similar to the above rates in each albuminuria group. There was also a correlation between age and pulse pressure in all albuminuria categories ($r=0.36$, $p<0.001$ in normo- and microalbuminuric groups and $r=0.27$, $p<0.001$ in macroalbuminuric group). Mean HbA1c values were similar among the albuminuria categories and the age groups.

Mean GFR was significantly lower in the group treated with AHT compared to the group not on AHT in the normoalbuminuria (83+2 vs 101+3 ml/min/1.73 m^2, $p<0.01$) and microalbuminuria (76+3 vs 101+7 ml/min/1.73 m^2, $p<0.01$) categories. However, the subjects on AHT were significantly older, had a longer disease duration and higher systolic blood pressure and pulse pressure. AHT was associated with a lower GFR even when adjusted for age.

Conclusions: This study shows a strong association between increasing age and GFR in Type 2 diabetes, above and beyond that expected with ageing alone which may be related to increasing pulse pressure with age. Similar decrements in GFR with age were observed in normo-, micro- and macroalbuminuric patients, suggesting that this apparent age related GFR decline is independent of the albuminuria status. However, as increased albumin excretion and low GFR are each related to increased cardiovascular risk, longitudinal studies free from possible survival bias are required to further delineate the true decline in GFR with ageing.

Figure: Mean GFR across age groups in Type 2 diabetic subjects



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Relationship between homocysteine and incipient diabetic nephropathy in type 2 diabetes mellitus

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Back ground and Aims: To investigate the ralationship betwee Homocysteine (Hcy)and incipient diabetic nephropathy in type 2 diabetes mellitus(T2DM),and explor the factors influencing plasma Hcy in cases of T2DM.

Materials and Methods: Two hundred and fifty-two cases of T2DM and 30 cases of normal control(CON)were enrolled in the study.The cases of T2DM were divided into two groups: one group without complications (NDC) and another group with incipient diabetic nephropathy(DN).Plasma Hcy, folic acids(FA),vitaminB_{12}($VitB_{12}$),HbA_1c,fasting blood glucose (FBG),postprandial blood glucose(PBG), creatinine(Cr),cholesterol(CHO),triglyceride(TG),transforming growth factor β_1 (TGF-β_1) levels,urinous N-acetylglucosaminidase(NAG) and microalbumin/creatinine (MA1b/cr) levels were measured.The mutation of the C677T transition of methylenetetrahydrofolate(MTHFR) was determined by PCR-RFLP.

Results: The patients with T2DM had higher Hcy levels and higher prevalence of hyperhomocysteinemia (Hhcy) than normal group,while incipient diabetic nephropathy higher than those without this complication.The Hcy levels of group NDC versus CON is (11.91±7.29) versus (9.72±2.20), group DN versus NDC is (16.60±12.59) versus (11.91±7.29), ($P<0.05$).The incidence rate of Hhcy in group DN is 45.4%, NDC is 22.9%, while CON is 3.3%,($P<0.05$). The spearman's correlation analysis between Hcy and other

variables showed that the Hcy levels in T2DM were positively related to TT genetype of MTHFR(r=0.654), BMI(r=0.214), FBG(r=0.141), PBG(r=0.171), HbA1c(r=0.170), NAG(r=0.178), MA1b/cr(r=0.3), course(r=0.150), smoking(r=0.166) and metformin treatment(r=0.157), (P<0.05), and was negtively related to plasma FA(r=−0.133) and $VitB_{12}$ levels (r=−O.273), (P<0.05). Multiple logistis regression analysis showed that course, diastolic blood preasure (DBP), FPG, NAG and plasma Hcy levels were the independent risk factors for incipient diabetic nephropathy, their OR value is 1.074, 1.054, 1.206, 1.043, 1.057, and P value is 0.045, 0.001, 0.003, 0.000 and 0.005 respectingly.

Conclusion: Hhcy was a independent risk for incipient diabetic nephropathy; TT genetype of MTHFR, FA, $VitB_{12,}$ MA1b/cr,NAG level and metabolic disorder influenced the plasma Hcy level in T2DM.

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Systemic inhibition of nitric oxide synthase unmasks differences in renal vascular function between African and Caucasian patients with type 2 diabetes without nephropathy

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Background and Aims: Systemic hypertension promotes renal disease and is associated with altered nitric oxide bioavailability. We considered that prior to the onset of nephropathy, variations in this pathway may be evident between racial groups with differing susceptibility diabetic microangiopathy.

Materials and Methods: We studied 18, age normotensive, normoalbuminuric patients with type 2 diabetes of African and Caucasian origin of similar age and duration of diabetes. Renal blood flow was measured using a constant infusion of paraminohippurate, in response to an amino acid mixture and N^g-monomethyl-l-arginine (L-NMMA) under euglycaemic conditions.

Results: There were no differences in basal renal plasma flow (RPF) rates, systolic or diastolic blood pressure in the African and Caucasians groups (533.7[174.7] vs 565.3 [260.8] mls/min/1.73 m^{-2}; p=0.78, 124.9[23.7] vs 121.6 [12.3] mmHg; p=0.29 or 77.1[9.5] vs 76.3[5.7] mHg; p=0.81). Addition of L-NMMA to the amino acid infusion resulted in a significant mean [95% confidence interval] increase in systemic blood pressure in the African (Δ 7.3 [1.0–13.7] mmHg; p=0.03) but not in the Caucasian group (Δ 2.4 [−3.5–8.3] mmHg;p=0.38). There were mean falls in RPF of 13.3 and 46.0 mls/min/1.73 m^{-2} (p<0.05 and p=0.25), whilst renal vascular resistance (RVR) was unchanged at 0.11 (0.02) mmHg/ml/min/1.73 m^{-2};p=0.61 but had risen to 0.14 (0.04) mmHg/ml/min/1.73 m^{-2}; p=0.036 in Caucasian and African groups respectively. Systemic mean arterial pressure was associated with RVR in the African (r^2=0.77; p=0.004) but not in the Caucasian group (r^2=0.03; p=0.68).

Conclusion: In these patients with type 2 diabetes without nephropathy, systemic inhibition of nitric oxide synthase caused a greater pressor response and decrement in renal blood flow in patients of African compared with Caucasian origin. This suggests that NO bioavailability plays a more critical role in preserving renal haemodynamic integrity in African than Caucasian patients. These observations may be relevant to the racial differences in vulnerability to diabetic renal disease.

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Prevalence of ischaemic nephropathy in type 2 diabetes

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Background and Aims: The aim of study was to evaluate the prevalence of renal artery stenosis (RAS) in subjects with type 2 diabetes and hypertensive aged 50 and above by using duplex sonography, to assess clinical, biochemical and hemodynamic predictors of ischemic nephropathy.

Materials and Methods: 156 patients (62 males and 94 females) with type 2 diabetes were studied by color-flow duplex sonography of renal artery and abdominal aorta.

Results: 55 (35.3%), cases had significant RAS (26.3% monolateral, 8.97% bilateral). Men more frequently had RAS (43.5% of all men, 29.8% of all female). 39.7% had diabetic retinopathy, 47.4% had hypertonic angioretinopathy. RAS was present in 41.3% of patients in the age group 50–59, 30.7% in the 60–69 and 40.9% at age 70 and above. RAS-negative and RAS-positive groups were similar in duration of diabetes, aged, hyperlipidemia, 24-h blood pressure, diabetic management and glomerular filtration rate (GFR). The comparison between patients with and without RAS revealed significant difference in the incidence of previous myocardial infarction (p=0.027), prevalence of renal dimensions asymmetry (>11 mm) (p=0.02), cardiac

interventricular septum hypertrophy (p= 0.05), hyperfibrinogenaemia (p=0.001), smoking (p=0.003).
Conclusions: Color-duplex ulnrasonography-detected RAS is common in type 2 diabetic patients (specially in smoking males) with myocardial dysfunction and rheological abnormalities.

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Metabolic control and insulin analogues influence erythropoietin levels in type 2 diabetic patients with nephropathy

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Background and Aims: Diabetic patients with nephropathy (N) show a disturbed synthesis of erythropoietin (EPO) even at moderate degree of kidney dysfunction compared to nondiabetic patients. Possible consequences are an earlier onset of anemia and/or cardiovascular complications, since EPO - aside from its stimulating influence on erythropoesis - posses protective effects on development of ischemic damage. There is only little information on factors which influence levels of erythropoetin in type 2 diabetic patients with and without nephropathy
Materials and Methods: The following parameters were determined in 248 type 2 diabetic patients (54% men, age<75 years) of our outpatient clinic: erythropeotin (EPO) levels, hemoglobin (Hb), calculated creatinine clearance (CCL), albumin excretion (AE), HbA1c, high sensitive CRP (hs CRP), NT-proBNP, serum lipids, kind of therapy. Patients were divided in two group:„without nephropathy“ (=N- ; CCL >90 ml/min, no increased AE; n=86) and„with nephropathy“ (=N+: CCL<90 ml/min with or without AE; n=148). Apparently healthy nondiabetic people served as controls (n=61).
Results: EPO levels were 10,9 U/l in nondiabetic C, 14.5 U/l in group N- and 13.0 U/l in group N+. In N-, EPO showed a negative correlation to Hb (r=−0,357; p<0,001), no corresponding correlation could be found in N+. Patients with poor metabolic control (HbA1c > 9%) exhibited lower EPO levels (12 U/l) compared to patients with better metabolic control (HbA1c<7%; 15 U/l) in both groups. In N- higher EPO levels were associated with betablocker therapy, in N+with insulin therapy, especially with insulin analogs. Positive correlation could be found between EPO and levels of NT-proBNP (r=0.324, p<0.002) and hsCRP (r=0,222; p<0.04). A multiple linear regression analysis showed that in group N- EPO levels were primarily influenced by hemoglobin concentration and betablocker therapy. In group N+important influencing factors were HbA1c - levels, use of insulin analogs and RAS- inhibiting substances.
Conclusion: EPO levels correlated with quality of metabolic control and kind of antidiabetic therapy in type 2 diabetics with nephropathy. These findings may have important therapeutic implications for the treatment of diabetic patients with renal insufficiency with respect to impaired synthesis of EPO.
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Haematocrit lowering effect and anaemia due to inactivation of renin-angiotensin system in type 2 diabetic patients with normal renal function

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Background and Aims: An intriguing interrelationship has been revealed between Renin Angiotensin System (RAS) and erythropoesis. Decrease in hematocrit (Hct) and anemia has been reported in patients with renal, cardiac and pulmonary diseases treated with Angiotensin Converting Enzyme Inhibitors (ACEI) and/or Angiotensin Receptor Blockers (ARBs). Anemia is a common finding in patients with diabetic nephropathy, and identifies a subset of high-risk patients for premature death. The aim of this study was to explore the Hct lowering effect of ACEI and/or ARBs in type 2 diabetic (T2D) patients with normal renal function.
Materials and Methods: From a cohort of 1750 pts with T2D followed in our tertiary diabetic clinic, we selected 180 patients (group Rx), with normal renal function (Cr≤1.5 mg %) treated with ACEI and/or ARBs. These patients where matched for age, gender, renal function and duration of T2D with 180 patients (group C) who did not received ACEI or ARBs. Exclusion criteria included: malignancy, chronic inflammation, hepatic disease, gastrointestinal blood loss, abnormal MCV or therapy with PPRA-γ agents. Hct and anemia (Hct<36% female/ Hct<39% male) where recorded in group Rx, prior to ACEI or ARBS therapy and 1 and 3 years after RAS inactivation. Corresponding values were also recorded in group C patients.
Results (Mean±SD): The rate of complications, were higher in group Rx than in group C: hypertension (91.6% vs. 23.9%, p=0.0001), hyperlipidemia (43.3% vs. 32.7%, p=0.039), protein in urine (9.4% vs. 1.1%, p=0.0001),

coronary artery disease (6.1% vs. 0.5%, p=0.03). This reflects the high-risk group, which warranted RAS inactivation. In fact patients in group Rx had greater RAS activation than group C, as indicated by ACE polymorphisms (DD/ID/II was 53%/38%/9% group Rx 33%/56%/11% group C, p=0.01). Glycocylated hemoglobin (HbA1c) values were comparable at baseline between the two groups (7.6±2 group Rx vs. 7.13±2.4 group C). HbA1c values remained stable in group C after 1 and 3 years of observation but decreased in group Rx (6.6±1.3 the 1st year, p=0.003, 6.7±1.4 the 3rd year, p=0.005).
Both Hct values and prevalence of anemia did not differ between the groups at baseline (Hct: 43.3±3.3% group Rx vs. 42.6±3.3% group C, and anemia: 5.3% group Rx vs. 3.4% group C). No significant difference in Hct and prevalence of anemia was observed in group C patients after 1 and 3 years of follow up. In contrast in group Rx Hct decreased (to 41.8±3.5%, p=0.003) after 1 year and remained unchanged for at least 3 years. Moreover, at the end of the 3rd year the prevalence of anemia in group Rx was higher than group C (7.9% vs. 2.8%, p=0.037).
Conclusion: Reduction in Hct is often found in diabetic patients with normal renal function, after treatment with ACEI and/or ARBs. Anemia is seen three times more often in patients treated with ACEI and/or ARBs compared to controls. Therefore the use of these drugs should be considered when other causes of anemia are excluded.

PS 92

Erythropoietin and nephropathy

1060

Human cell line derived erythropoietin (epoetin delta) administered subcutaneously is effective in the management of anaemia associated with chronic kidney disease

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Background and Aims: Epoetin delta (Shire Pharmaceuticals) is an erythropoietin produced by gene-activation technology in a human cell line and has a human-type carbohydrate profile, unlike recombinant erythropoietins produced in Chinese Hamster Ovary cell lines. In this study (carried out between 1998–2000), we assessed the efficacy and safety of subcutaneous (sc) epoetin delta in patients with chronic kidney disease (CKD) and anaemia.
Materials and Methods: Patients with CKD who had previously received sc epoetin for at least 30 days and had a haemoglobin level between 9.6 and 10.4 g/dL at baseline, were eligible for this multicentre, open-label study. Patients who entered the study received sc epoetin delta at an initial dose identical to their previous epoetin treatment for up to 52 weeks. Treatment was given one, two or three times per week (depending on previous epoetin regimen) and was titrated to maintain haemoglobin levels at 10.0–12.0 g/dL. Primary objective was to assess mean haemoglobin levels over Weeks 12, 16, 20 and 24 in the study population who had at least one haemoglobin measure during this period.
Results: In total, 478 patients entered the study, 411 (288 on haemodialysis, 91 peritoneal dialysis and 32 who did not require dialysis) of whom were included in the primary efficacy analysis. Diabetic nephropathy was one of the most prevalent primary causes of CKD (23.6% of patients). Most patients (319, 66.7%) completed the full 52 weeks of treatment. The most common reason for withdrawal was 'other' (38 patients, 7.9%). One specific reason for this was a sterility test failure of one batch of study drug. As no immediate replacement batch was available, 19 patients were withdrawn and the study was terminated in the UK. Subsequent investigation found no contamination. Requirement for red blood cell transfusion (a per protocol withdrawal event) was the next most common withdrawal reason (27 patients, 5.6%). The mean±SD haemoglobin level over Weeks 12, 16, 20 and 24 was 11.31±1.11 g/dL. Haemoglobin levels > 10.0 g/dL were recorded in 83.9% of patients over this period. Mean haemoglobin levels were > 11.0 g/dL in all analysed subgroups (e.g. dialysis requirements, age, sex, race). Mean dose of epoetin delta was 45.2 IU/kg and there was no evidence that dosing frequency had an effect on efficacy (mean haemoglobin levels, three-times weekly *vs.* once weekly: 11.16±1.09 g/dL *vs.* 11.50±1.16 g/dL). Control of anaemia was maintained over the 52 weeks of the study, with mean haemoglobin levels from Week 37 to Week 52 of 11.16±1.14 g/dL. Overall, 86.8% of patients experienced at least one adverse event during the study, with 12.3% of patients having an event that was considered possibly related to treatment. The most common event thought possibly related to treatment was hypertension. Serious adverse events occurred in 49.4% of patients, with 4.6% of patients experiencing a serious event that was thought possibly related to treatment.

Conclusion: Subcutaneous epoetin delta is effective in the management of patients with anaemia resulting from CKD, many of whom have diabetic nephropathy. It is effective in maintaining haemoglobin levels at 10.0–12.0 g/dL regardless of dialysis requirements and can maintain haemoglobin control at a dosing frequency of once per week. The adverse event profile was as expected in this patient group.
Supported by Hoechst Marion Roussel

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Epoetin delta, erythropoietin produced by a human cell line, is as effective as epoetin alfa in patients with renal anaemia, including those with diabetic nephropathy

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Background and Aims: Recombinant erythropoietins are used extensively in the management of anaemia associated with chronic kidney disease. At present, all of these agents are produced in Chinese Hamster Ovary (CHO) cell lines. Epoetin delta is an erythropoietin produced by gene-activation technology in a human cell line.
Materials and Methods: In this double-blind, active-comparator study, patients requiring haemodialysis and previously receiving intravenous erythropoietin therapy were randomized in a 3:1 ratio to receive epoetin delta or epoetin alfa. Eligible patients (haemoglobin level at baseline: 9.6–12.4 g/dL) received a starting dose equivalent to that they were receiving before randomization, given intravenously three-times per week. Doses were titrated to maintain haemoglobin at 10–12.0 g/dL. The double-blind treatment lasted for 24 weeks and was followed by a 28-week, open-label phase during which all patients received epoetin delta. Primary efficacy endpoint was to assess the difference in mean haemoglobin levels (over Weeks 12, 16, 20 and 24) between the two groups. Subgroup analyses included patients with diabetic nephropathy.
Results: In total, 752 patients were randomized of whom 666 (363 men, 303 women) had a recorded baseline haemoglobin value and at least one haemoglobin measurement between Weeks 12 and 24. The epoetin delta group included 491 patients (mean age: 56.3±15.6 years), with 175 in the epoetin alfa group (mean age: 56.8±15.1 years). Primary diagnosis was diabetic nephropathy in 29.5% of the epoetin delta group and in 37.1% of the epoetin alfa group. The double-blind period was completed by 583 patients (77.5%) and all of these entered the open-label phase. Mean haemoglobin levels between Weeks 12 and 24 were 11.57 g/dL (95% CI: 11.47–11.66) and 11.56 g/dL (95% CI: 11.41–11.70) in the epoetin delta and epoetin alfa groups, respectively. The difference between the two groups was 0.01 g/dL (90% CI: -0.13–0.15) demonstrating equivalence between the two study agents. Mean dose was similar in the two groups. Analysis of results from Week 12 to study completion showed that epoetin delta maintained control of haemoglobin, with a mean level of 11.31 g/dL. Similar efficacy results were seen in the subgroup of patients with diabetic nephropathy (mean haemoglobin levels Weeks 12–24, epoetin delta *vs.* epoetin alfa: 11.47 g/dL *vs.* 11.47 g/dL). Adverse events possibly related to treatment were reported in 9.2% of patients receiving epoetin delta and 8.4% of those receiving epoetin alfa. Serious adverse events occurred in 47.3% of patients (1.7% thought possibly related to treatment) receiving epoetin delta over 52 weeks of treatment and in 33.0% of patients receiving epoetin delta over 24 weeks. The latter value compared with 26.7% in the epoetin alfa group over the same period. No deaths were thought to have any possible relation to study treatment. The overall frequency of adverse events was similar in diabetic and non-diabetic patients.
Conclusion: Epoetin delta is effective in maintaining haemoglobin levels of 10–12 g/dL over 24 weeks in patients requiring haemodialysis (including patients with diabetic nephropathy), and is able to maintain control of haemoglobin over 52 weeks. Results are similar to those with epoetin alfa.
Supported by Hoechst Marion Roussel

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Anaemia correction with epoetin beta in patients with diabetes and chronic kidney disease - primary results of the Anaemia CORrection in Diabetes (ACORD) study

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Background and Aims: Diabetes is a major risk factor for cardiovascular (CV) disease, with the risk amplified by the presence of diabetic nephropathy (DN) and anaemia. Anaemia is associated with the incidence of CV events and a predictor for left ventricular hypertrophy. ACORD is the first prospective study examining whether attaining a target Hb of 13–15 g/dL in patients with early diabetic nephropathy and moderate anaemia can reduce CV risk, preserve renal function, improve clinical outcomes and quality of life (QoL).
Material and Methods: ACORD was an open-label, multi-centre, randomised trial in patients with diabetes and stage II-III chronic kidney disease (CKD) comparing the effect of epoetin beta treatment to attain a target Hb of 13–15 g/dL (Group 1 -G1), *vs* maintaining a Hb range of 10.5–11.5 g/dL (Group 2 - G2). The primary endpoint was change from baseline in left ventricular mass index (LVMI) at 15 months. Additional echocardiographic and clinical variables, renal function and quality of life (QoL) were also examined.
Results: A total of 170 patients were randomized into two treatment arms (G1: 88; G2: 82). Study groups were well matched at baseline. The majority of patients had diabetic retinopathy (G1: 84%; G2: 83%) and pre-existing CV disease (G1: 90%; G2: 94%). Mean age was 56 years, HbA1c 8.1%, systolic and diastolic blood pressure (SBP/DBP) 131/76 mmHg. Change in Hb from baseline to study end differed significantly between study groups in accordance with the relevant target windows (G1: 11.8 to 13.4 g/dL; G2: 11.7 to 11.9 g/dL; $p<0.001$) supporting the efficacy of once weekly SC epoetin beta treatment. At study end there was no significant difference in LVMI ($p=0.8811$) between study groups. Multivariate analysis, however, showed a trend ($p=0.1539$) towards a reduction in LVMI in G1 compared to G2. Secondary echocardiographic variables did not differ between treatment groups. Anaemia correction with epoetin beta was safe without clinically significant difference between G1 and G2. Blood pressure (BP) was well controlled during the study with no difference in mean BPs at study end (SBP/DBP in G1: 136/75 mmHg; G2: 134/77 mmHg). The annual CV event rate was low (~4%). There were significant benefits in QoL in favour of G1 (by SF-36; $p=0.039$). There was no difference in renal disease progression between study groups. Mean creatinine clearance decreased by 3.8 mL/min/yr in G1 and 3.9 mL/min/yr in G2.
Conclusion: In diabetic patients with early DN and anaemia once weekly treatment with SC epoetin beta resulted in a significant increase in Hb and related significant improvements in quality of life. In contrast to clinical expectation, LVMI was maintained stable in both study groups with no difference in favour of higher Hb targets, despite a trend towards regression of LVMI in multivariate analysis. Treatment with epoetin beta to avoid severe anaemia was safe. In this study the annual CV event rate was considerably lower than reported in previous studies.
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The influence of erythropoietin on immunity in patients with diabetic nephropathy and anaemia

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Background and Aims: The biological effect of erythropoietin (EPO) is more widely. Erythropoietin not only restores erythropoiesis in patients with EPO deficiency anemia but improves the quality of life and survival in patients with cardiovascular disease. The influence of EPO on the other systems is not well known. The aim of this study was to investigate the influence of EPO on immunity in patients with diabetic nephropathy (DN) treated with recombinant [r]EPO.
Materials and Methods: Patients with DN and EPO deficiency anemia and those with DN without anemia were examined. Control group was consisted of age- and sex-matched healthy persons. Fasting plasma endogen [e]EPO levels were measured using ELISA. Hemoglobin (Hb), erythrocyte (RBC), mean content of hemoglobin (MCH), mean concentration hemoglobin in erythrocyte (MCHC), mean volume erythrocyte (MCV) were measured with hematological analyzer (Coulter). Lymphocyte (CD3+, CD4+, CD8+, CD20+, and CD56+cells) immunophenotype was determined by the method of flow cytometry on FACStar Plus. rEPO-beta (Roche) was injected subcutaneously at a dose of 2000 IU twice a week.
Results: Our data are shown in table. The level of eEPO in patients with DN and anemia was significant lower compared with patients with DN without anemia and healthy subjects. However, there was no direct correlation between degree of anemia and plasma eEPO level. Absolute content of CD3+, CD4+, CD56+cells was decreased in DN patients with and without anemia before treatment. Anaemia was significantly improved after 8 weeks of rEPO administration, there were no significant differences in MCV, MCH, MCHC before and after

treatment. At the same time absolute content of CD3+, CD4+, CD56+cells and CD4/CD8 index normalized.

Characteristic	Control group	Patients with DN without anemia	Patients with DN and anemia	
			Before treatment	After treatment
n	30	30	20	20
eErythropoietin	13,48±2,08	9,92±1,03*	3,82±0,94*	
Hb(g/dl)	14,2±0,31	13,1±0,29	7,51±0,28	8,85±0,39**
RBC (10^{12}l)	4,7±0,41	4,2±0,37	2,59±0,10	3,24±0,35**
Ht %	39,0±1,28	33,1±1,29	24,4±1,17	32,33±1,24**
Lymphocyte (10^9l)	2,12±0,08	1,83±0,1	1,39±0,09	1,62±0,10
CD3+cells (10^9l)	1,39±0,09	1,07±0,05	1,02±0,05	1,31±0,06**
CD4+cells (10^9l)	0,98±0,04	0,73±0,03	0,65±0,04	0,88±0,05**
CD8+cells (10^9l)	0,48±0,04	0,42±0,03	0,39±0,03	0,40±0,04
CD20+cells (10^9l)	0,25±0,02	0,24±0,02	0,19±0,01	0,18±0,02
CD56+cells (10^9l)	0,29±0,02	0,21±0,02	0,18±0,02	0,25±0,02**
CD4/CD8 index	1,98±0,1	1,73±0,1	1,66±0,1	2,20±0,09**

*p<0,05 for difference between control group and diabetic patients with and without anemia

**p<0,05 for difference between patients with DN and anemia before and after treatment

Conclusion: We showed for the first time that increase of the main parameters of erythropoiesis in patients with DN and anemia leads to normalization parameters of innate and acquired immunity, improves the quality of patients' life.

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In patients with diabetes on dialysis, subcutaneous C.E.R.A. (continuous erythropoietin receptor activator) maintains Hb levels when administered up to once monthly

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Background and Aims: Chronic kidney disease (CKD) is a major complication of diabetes, and around 70–80% of patients with CKD have anaemia. C.E.R.A., an innovative agent with unique erythropoietin receptor activity and a prolonged half-life, is currently in development to provide correction of anaemia and stable control of haemoglobin (Hb) levels at extended administration intervals in patients with CKD on dialysis and not on dialysis. The present study examined the efficacy of subcutaneous (SC) C.E.R.A. in maintaining Hb levels at administration intervals up to 1×/4 wk in patients on dialysis with and without diabetes who were previously maintained on SC epoetin alfa or beta 1–3×/wk. The effect of the switch in therapy on Hb levels was compared in patients with and without diabetes.

Material and Methods: This large Phase III, multicentre, open-label study included dialysis patients ≥18 years of age receiving stable SC epoetin alfa or beta (1–3×/wk) maintenance therapy. Patients received adequate dialysis and had stable baseline Hb (10.5–13.0 g/dL) and adequate iron status. After a 4-week baseline period, patients (n=572) were randomised (1:1:1) to receive SC C.E.R.A. 1×/2 wk (n=190) or 1×/4 wk (n=191) or to continue the same SC epoetin regimen (1–3×/wk; n=191) for 52 weeks. Dosage was adjusted to maintain Hb within±1.0 g/dL of the baseline level and between 10.0–13.5 g/dL. Here we present an analysis of change in mean Hb levels between baseline and the evaluation period (weeks 29–36) according to diabetic status.

Results: Patients with/without diabetes were randomised to each treatment group as follows: 62/128 (C.E.R.A. 1×/2 wk), 52/138 (C.E.R.A. 1×/4 wk) and 63/126 (epoetin). Mean Hb levels at baseline were similar across treatment groups and in patients with and without diabetes: C.E.R.A. 1×/2 wk (11.8 and 11.7 g/dL, respectively); C.E.R.A. 1×/4 wk (11.7 and 11.6 g/dL); epoetin (11.6 and 11.7 g/dL). During the evaluation period, mean Hb levels in patients with and without diabetes were: C.E.R.A. 1×/2 wk (11.8 and 11.7 g/dL, respectively); C.E.R.A. 1×/4 wk (11.7 and 11.3 g/dL); epoetin (11.5 and 11.4 g/dL). Hb was maintained between the baseline and evaluation periods in all treatment groups and in patients with and without diabetes. Mean changes in Hb between baseline and the evaluation period (weeks 29–36) in patients with and without diabetes were: C.E.R.A. 1×/2 wk (−0.38 and −0.06 g/dL, respectively); C.E.R.A. 1×/4 wk (−0.19 and −0.30 g/dL); epoetin (−0.04 and −0.24 g/dL). C.E.R.A. was well tolerated, with a safety profile characteristic of the patient population. The most frequent adverse events were hypertension, procedural hypotension and nasopharyngitis.

Conclusion: These Phase III results indicate that SC C.E.R.A effectively maintained stable Hb levels in dialysis

patients who converted from epoetin 1–3×/wk to C.E.R.A. 1×/2 wk or 1×/4 wk, irrespective of diabetic status. Maintaining adequate Hb within guideline levels is an important goal of anaemia management. The possibility of achieving this with C.E.R.A. administered at extended intervals up to once monthly could provide benefits for both patients and healthcare providers.
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Hb levels in patients with diabetes and chronic kidney disease receiving dialysis are maintained with C.E.R.A. (Continuous Erythropoietin Receptor Activator) administered once monthly

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Background and Aims: Chronic kidney disease (CKD) is a major consequence of diabetes and diabetes is the leading cause of CKD in many countries. Anaemia is often associated with both diabetes and CKD and anaemia is often more severe in patients with diabetes than in those without diabetes at similar levels of kidney function. C.E.R.A., an innovative agent with unique erythropoietin receptor activity and a prolonged half-life, is currently in development to provide correction of anaemia and stable control of haemoglobin (Hb) levels at extended administration intervals in patients with CKD on dialysis and not on dialysis. Here, we present pooled data from two Phase III studies in dialysis patients where C.E.R.A. 1×/4 wk was administered either by subcutaneous (SC) or intravenous (IV) injection. The efficacy of C.E.R.A. in maintaining Hb levels in patients who converted from epoetin 1–3×/wk directly to C.E.R.A. 1×/4 wk in patients with and without diabetes who were previously maintained on IV or SC epoetin alfa or beta was evaluated.
Material and Methods: Two large Phase III, multicentre, open-label studies were conducted in dialysis patients ≥18 years of age receiving stable epoetin alfa or beta (1–3×/wk) maintenance therapy. Patients with adequate dialysis and iron status who had stable baseline Hb (10.5–13.0 g/dL) were included. After a 4-week baseline period, patients (n=1245) were randomised (1:1:1) to receive IV or SC C.E.R.A. 1×/2 wk (n=413) or 1×/4 wk (n=415) or to continue the same IV or SC epoetin regimen (1–3×/wk; n=417) for 52 weeks. Dosage was adjusted to maintain Hb within±1.0 g/dL of the baseline level and between 10.0–13.5 g/dL. Here we present an analysis of Hb data stratified by the presence or absence of diabetes. The primary endpoint was the mean change in Hb between baseline and the evaluation period (weeks 29–36).
Results: 144/415 (35%) of the patients randomised to the C.E.R.A. 1×/4 wk group were diabetic. Mean Hb levels at baseline in the C.E.R.A. 1×/4 wk group were similar in patients with and without diabetes: 11.8 and 11.7 g/dL, respectively. During the evaluation period, mean Hb levels in patients with and without diabetes were also similar: 11.6 and 11.5 g/dL, respectively. C.E.R.A. 1×/4 wk maintained Hb between the baseline and evaluation periods in patients with and without diabetes. Mean Hb changes during this period were −0.23 and −0.21 g/dL in diabetic and non-diabetic patients, respectively. C.E.R.A. was well tolerated, with a safety profile characteristic of the patient population. The most frequent adverse events were hypertension, nasopharyngitis and diarrhoea.
Conclusion: These pooled data from two large Phase III studies where C.E.R.A was administered either IV or SC indicate that stable Hb levels were maintained in dialysis patients with or without diabetes who converted directly from epoetin 1–3×/wk to C.E.R.A. 1×/4 wk. The feasibility of maintaining adequate Hb levels within guideline ranges with C.E.R.A. administered once monthly could offer potential benefits to patients regardless of their diabetic status.
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Intravenous C.E.R.A. (continuous erythropoietin receptor activator) administered up to once monthly maintains Hb levels in patients with diabetes on dialysis

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Background and Aims: Anaemia often manifests early in the course of chronic kidney disease (CKD) in patients with diabetes. C.E.R.A., an innovative agent with unique erythropoietin receptor activity and a prolonged half-life, is currently in development to provide correction of anaemia and stable control of haemoglobin (Hb) levels at extended administration intervals in patients with CKD on dialysis and not on dialysis. In the present study, we examined the efficacy of intravenous (IV) C.E.R.A. in maintaining Hb levels at administration intervals up to once

monthly in patients on dialysis with and without diabetes previously maintained on IV epoetin alfa or beta 1–3×/wk. The effect of the switch in therapy on Hb levels was compared in patients with and without diabetes.

Material and Methods: This large Phase III, multicentre, open-label study included dialysis patients ≥18 years of age receiving stable IV epoetin alfa or beta (1–3×/wk) maintenance therapy. Patients received adequate dialysis and had stable baseline Hb (10.5–13.0 g/dL) and adequate iron status. After a 4-week baseline period, patients (n=673) were randomised (1:1:1) to receive IV C.E.R.A. 1×/2 wk (n=223) or IV C.E.R.A. 1×/4 wk (n=224) for 52 weeks or to continue the same IV epoetin regimen (1–3×/wk; n=226). Dosage was adjusted to maintain Hb within±1.0 g/dL of the baseline level and between 10.0–13.5 g/dL. Here we present an analysis of change in mean Hb levels between baseline and the evaluation period (weeks 29–36) according to diabetic status.

Results: Patients with/without diabetes were randomised as follows: 107/116 (C.E.R.A. 1×/2 wk), 92/132 (C.E.R.A. 1×/4 wk) and 114/112 (epoetin). Mean Hb levels at baseline were similar across treatment groups and in patients with and without diabetes: C.E.R.A. 1×/2 wk (12.0 g/dL for both groups); C.E.R.A. 1×/4 wk (11.9 and 11.8 g/dL); epoetin (11.8 and 12.0 g/dL). At the end of the evaluation period (week 36), mean Hb levels in patients with and without diabetes were: C.E.R.A. 1×/2 wk (11.7 and 11.9 g/dL, respectively); C.E.R.A. 1×/4 wk (11.5 and 11.8 g/dL); epoetin (11.7 and 11.8 g/dL). Hb was maintained between the baseline and evaluation periods in all treatment groups and in patients with and without diabetes. Mean changes in Hb between baseline and the evaluation period (weeks 29–36) in patients with and without diabetes were: C.E.R.A. 1×/2 wk (−0.36 and −0.08 g/dL, respectively); C.E.R.A. 1×/4 wk (−0.25 and −0.11 g/dL); epoetin (−0.13 and −0.32 g/dL). C.E.R.A. was well tolerated, with a safety profile characteristic of the patient population. The most frequent adverse events were diarrhoea, nasopharyngitis and hypertension.

Conclusion: These Phase III results indicate that IV C.E.R.A. effectively maintained stable Hb levels in dialysis patients who converted from epoetin 1–3×/wk to C.E.R.A. 1×/2 wk or 1×/4 wk irrespective of the presence of diabetes. Adequate maintenance of Hb levels within guideline ranges is an important goal of anaemia management. The possibility of achieving this with C.E.R.A. administered at extended intervals up to once monthly could provide benefits for both patients and healthcare providers.

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Nephropathy - experimental

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Increased glomerular cell (podocyte) apoptosis in rats with streptozotocin-induced diabetes mellitus: relation to increased p53 expression

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Background and Aims: Glomerular cell apoptosis is considered as a major mechanism for resolution of glomerular/mesangial hypercellularity, but it may also play a pathogenic role in glomerular injury by promoting tissue repair leading to matrix deposition. This study was aimed at assessing glomerular cell death rate and time course in experimental diabetic nephropathy and to identify the cell type(s) and the molecular mechanisms(s) involved, in parallel with renal/glomerular structural changes and expression of apoptosis-related proteins in experimental diabetes.

Materials and Methods: Male Sprague-Dawley rats were rendered diabetic (D) with streptozotocin (55 mg/kg) and killed 2, 4 or 6 months later, together with age-matched nondiabetic controls (ND). Apoptosis was assessed by immunohistochemistry for active caspase-3, followed by staining for specific glomerular cell type markers or counterstaining with PAS, renal/glomerular structure by morphometric analysis, the glycoxidation product Nε-carboxymethyllysine (CML), the AGE-receptors RAGE and galectin-3, p21 and phosphorylated p53 (in serine 15) by immunohistochemistry and p53 mRNA levels by competitive RT-PCR.

Results: Mean glomerular area, fractional mesangial area and glomerular sclerosis index increased progressively with age in both groups and were significantly higher ($p<0.001$) in D vs. ND (at 6 months, 17008±822 vs. 13384±710 μm^2; 30.7±3.1 vs. 18.4±1.4% and 1.2±0.2 vs. 0.5±0.1, respectively). Glomerular cell number and density decreased with age and were significantly lower in D vs. ND (70.0±7.1 vs. 80.9±5.8 cells/glomerulus, $p<0.01$, and 4.1±0.5 vs. 6.1±0.5 $1/mm^2$, $p<0.001$, respectively, at 6 months). This was accounted for by a progressive increase in glomerular cell apoptosis, which was significantly higher in D vs. ND rats, particularly at 6 months (1.5±0.4 vs. 0.3±0.1%, $p<0.001$). Apoptosing cells were mainly podocytes, though endothe-

lial and mesangial cells were not spared; moreover, the expression of the podocyte marker synaptopodin was markedly reduced in D vs. ND already at 2 months of disease duration, when apoptosis was only slightly increased, and further decreased thereafter. In glomeruli from D rats, CML, RAGE and galectin-3 were progressively upregulated, whereas p21 showed a peak at 4 months and phosphorylated p53 became detectable at 6 months; mRNA levels for p53 also increased progressively in D rats (+78% vs. ND, $p<0.001$ at 6 months).

Conclusion: These data indicate that, in parallel with the development of glomerular damage, significant apoptosis occurs in glomeruli from experimental diabetic rats, particularly in podocytes, and correlates with increased expression and activation of proapototic protein p53. Apoptosis is preceded and accompanied by a reduced expression of podocyte-specific proteins.

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Diabetes-induced up-regulation of uncoupling protein-2 in the kidney cortex results in increased oxygen consumption unrelated to active transport

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Background and Aims: Up-regulation of uncoupling protein 2 (UCP-2) is a possible protective mechanism against hyperglycaemia-induced mitochondrial formation of reactive oxygen species (ROS), the latter believed to be crucial for the development of diabetic nephropathy. This study evaluated the renal UCP-2-levels in control and streptozotocin-induced diabetic rats, and related this to changes in oxygen consumption.

Materials and Methods: UCP-2 in renal cortex and medulla were detected by Western Blot. Oxygen consumption was measured *in vitro* using isolated renal tubular cells placed in a sealed chamber and the oxygen consumption measured as the disappearance of oxygen corrected for protein content. Ouabain was used to inhibit transport-related oxygen consumption.

Results: UCP-2 protein levels (normalized to β-actin) in the renal cortex were increased 3.7-fold in diabetic animals compared to controls (0.56±0.08; n=5 vs. 0.15±0.05; n=5), and in the medulla the levels were increased 3-fold (0.39±0.09; n=5 vs. 0.13±0.04; n=5). Total oxygen consumption by isolated cortical cells from diabetic animals was not significantly different compared to controls (24.3±4.3; n=6 vs. 20.6±2.5 nmol/min/mg; n=5). However, oxygen consumption not related to active transport (i.e. ouabain-insensitive) was increased 1.7-fold in cells from the diabetic animals (13.1±1.9; n=6 vs. 7.5±1.4; n=5).

Conclusion: Diabetic animals show an increase in ouabain-insensitive oxygen consumption. Concomitantly, the diabetic animals have up-regulation of UCP-2 protein levels, suggesting that the increased oxygen consumption is due to increased mitochondrial uncoupling. The increased UCP-2 levels might be a protective mechanism against hyperglycaemia-induced mitochondrial ROS formation which warrants further studies.

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Diabetes-induced hyperfiltration in adenosine A1-receptor deficient mice lacking the tubuloglomerular feedback mechanism

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Background and Aims: Glomerular hyperfiltration is commonly found in diabetic patients early after the onset of the disease. This is also one of the first indications of the development of progressive diabetic nephropathy. It has been proposed that glomerular hyperfiltration is caused by a decreased delivery of electrolytes to the macula densa due to the increased sodium and glucose reabsorption in the proximal tubule, which would increase glomerular filtration rate (GFR) via the tubuloglomerular feedback (TGF) mechanism. In this study, we investigated the role of TGF in the development of diabetes-induced glomerular hyperfiltration by inducing diabetes in adenosine A1-receptor knock-out ($A1AR^{-/-}$) mice lacking the TGF and wild-type ($A1AR^{+/+}$).

Materials and Methods: Diabetes was induced by a single dose of alloxan (75 mg/kg bw) injected into the tail vein. The 24-hour urinary electrolyte excretion was measured in metabolism cages, GFR was determined by inulin clearance under isoflurane-anesthesia and histological changes were evaluated.

Results: All alloxan-treated animals developed a sustained hyperglycemia (≥20 mM). The diuresis was increased about 8-fold in both diabetic groups compared to normoglycemic controls. Normoglycemic animals had similar GFR independent of genotype ($A1AR^{+/+}$ 9.3±0.5, n=10 vs. $A1AR^{-/-}$ 10.1±0.8 μl/min/g bw, n=5) and the onset of diabetes

resulted in the development of similar glomerular hyperfiltration in both genotypes ($A1AR^{+/+}$ 14.0±1.7, n=9 vs. $A1AR^{-/-}$ 15.3±1.9 μl/min/g bw, n=6; $p<0.05$ vs. corresponding normoglycemic control group). The glomerular area was similar in both genotypes, and was unaltered by the onset of diabetes.

Conclusion: This study shows that the $A1AR^{-/-}$ mice develop diabetes-induced glomerular hyperfiltration, suggesting that the TGF mechanism is not involved in the development of hyperfiltration. Furthermore, the hyperfiltration in the present study was not related to alterations in the glomerular filtration area.

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Renal RhoA activity in experimental diabetes (DM)

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Introduction: RhoA is a member of Ras family of G-proteins involved in postreceptor signaling of growth factors, cytokines, and vasoactive hormones. Rho activation is dependent on its binding to GTP and subsequent translocation to the plasma membrane. Rho activation has been associated with growth signaling, cytoskeleton reorganization and cell contractility, and implicated in the pathophysiology atherosclerosis, hypertension and nondiabetic glomerulosclerosis (GS). However, the effects of DM and its treatment on renal RhoA have not been elucidated.

Methods: RhoA protein expression (western blotting) was determined in crude membrane and cytosolic fractions of renal cortical homogenates obtained from three groups of streptozotocin-diabetic rats (60 mg/kg, n=6/group) after 4-weeks duration of DM, and different metabolic control achieved by variable insulin treatment: severe hyperglycemia (DM-S, no insulin treatment); moderate hyperglycemia (DM-M, 4 IU of insulin/day); and tight metabolic control (DM-I, 12 IU/day). Renal RhoA localization was determined by immunohistochemistry (IHC). Data in diabetic rats were compared to age-matched non-diabetic controls (C). To elucidate effects of insulin per se on renal RhoA activity, further studies compared RhoA activation in C rats and in non-diabetic rats treated with 6 IU of insulin/day (C-I).

Results: Membrane RhoA expression was increased in all groups of diabetic rats (+50–90%, $p<0.01$ vs. C), whereas a decrease in cytosolic RhoA was observed in DM-S (−35%, $p<0.01$ vs. C). However, the ratio of membrane/cytosolic fractions, determined as the major characteristic of RhoA activity was markedly increased in all groups of diabetic rats irrespective of level of metabolic control (DM-S +767%; DM-M+654%; DM-I+491%; $p<0.01$ vs. C). Similar to DM rats, C-I demonstrated enhanced membrane RhoA translocation (+230%, $p<0.01$) as compared to C. IHC localized RhoA into the glomeruli, in particular in podocytes, and in luminal aspects of proximal tubules.

Conclusion: Early stages of diabetic nephropathy are associated with renal RhoA activation, which is not ameliorated by improved metabolic control, possibly due to independent effects of insulin. Glomerular localization of RhoA suggests its role in the development of proteinuria in DM.

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Reduced nitric oxide in the renal medulla of streptozotocin-induced diabetic rats is due to limited availability of arginine. A crucial role for hepatic cationic amino acid transporter-2A?

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Background and Aims: Altered concentrations of reactive oxygen species (ROS) and nitric oxide (NO) are suggested to mediate the development of diabetic nephropathy. We hypothesized that diabetes-induced generation of ROS changes renomedullary NO concentration, which alters local oxygen tension (PO_2) and blood flow.

Materials and Methods: Control and streptozotocin-induced diabetic animals, with or without α-tocopherol treatment, were administered L-arginine followed by L-NAME. NO concentrations were measured by haemoglobin-trapping using microdialysis probes, PO_2 by microsensors, blood flow by laser-Doppler flowmetry and plasma arginine and asymmetric dimethylarginine (ADMA) by HPLC. Liver arginase activity was measured *in vitro*. Cationic amino acid transporter (CAT)-2A and renal cortical CAT-1 mRNA levels were determined by real time PCR.

Results: Renomedullary NO concentrations were lower in diabetic animals (59±4 nmol/l, n=10) compared to control animals (122±7 nmol/l, n=8), and unaffected by α-tocoph-

erol. L-arginine increased renomedullary NO in diabetic animals, and totally restored NO in those fed α-tocopherol. L-arginine selectively increased medullary PO_2 in diabetics, although the blood flow increased equally in all groups. Diabetes decreased plasma arginine (−49%) and ADMA (−15%) independently of treatment. No differences were detected in either hepatic arginase activity or mRNA levels of CAT-1 in the kidney cortex. However, hepatic CAT-2A mRNA levels were increased about 3.5-fold in both diabetic groups.

Conclusion: We conclude that the reduced renomedullary NO concentration in diabetic animals is due to reduced arginine availability. ROS inhibition augments the NO response to arginine administration, but does not restore basal NO deficiency per se. The reduced plasma levels of L-arginine correlated with increased expression of hepatic CAT-2A, suggesting increased hepatic uptake of L-arginine. Increased NO concentration following L-arginine administration increases local PO_2 in diabetic animals. These changes may be important factors for the onset and progression of diabetes-induced renal dysfunction.

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p38 Mitogen-Activated Protein Kinase (MAPK) expression and activity in early and advanced diabetic nephropathy in rats

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Background and Aims: Studies have suggested that diabetes is associated with activation of p38 MAPK in the kidney. This alteration may have impact on renal morphology and function in diabetes. Available experimental evidence has suggested that p38 activation is a feature of early nephropathy in STZ-diabetic rats. However, whether p38 activation could be detected in advanced stages of the disease associated with proteinuria and glomerulosclerosis remains to be elucidated. In the present studies, we determined renal cortical p38 activity and expression in a rat model of Type 1 diabetes with early and advanced nephropathy.

Methods: Streptozotocin-diabetic rats (DM, 60 mg/kg bwt) were studied 4 weeks (n=8) and 12 months (n=4) after induction of DM. Diabetic rats were treated with suboptimal dose of insulin (2–4 U/day) to achieve moderate hyperglycemia. Age-matched non-diabetic rat were studied as controls (C). Renal cortical activity of p38 (*in vitro* kinase pull-down assay with ATF-2 used as a substrate), and protein expression of phosphorylated (active) and total p38 (P-p38,t-p38) were determined by western blotting in nuclear-free cortical homogenates. Renal localization of active p38 was determined by immunohistochemistry.

Results: Compared with age-matched controls, 4-week diabetic rats demonstrated increases in renal cortical p38 activity (81±19%, $p<0.05$), as assessed by *in vitro* kinase assay. Furthermore, expression of active p38 and P-p38/total p38 ratio, determined as another indicator of p38 activity, were also increased DM rats (P-p38 expression: 70±26%; P-p38/t-p38: 40±19%, $p<0.05$ vs. C). There were no differences in t-p38 between DM and C rats. In all rats, P-p38 was localized in macula densa (MD). In addition, DM rats had P-p38-positive cells in adjacent portions of the distal tubule and in glomeruli.

After 12 months of diabetes, the rats developed markedly elevated albuminuria and substantial glomerulosclerosis. Similar to 4-week diabetic rats, p38 kinase activity assay and determination of expression of P-p38 revealed marked increases in these parameters in DM as compared to C rats (p38 activity: 79±20%; P-p38 expression: 60±17%; P-p38/t-p38: 148±41%; $p<0.01$). Immunohistochemical localization of active p38 showed similar distribution of the protein in the renal cortex as observed in 4-week diabetic rats with most intensive stain found in macula densa cells and in distal tubules. However, no glomerular P-p38 immunoreactivity was detected in diabetic rats with established nephropathy.

Conclusions: Increases in renal cortical p38 MAPK activity are detectable not only early after onset of experimental diabetes, but also in diabetic rats with established nephropathy. Active p38 is predominantly localized in macula densa and distal tubules throughout the disease, whereas glomerular P-p38 is detectable only at early stages of diabetes.

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Deranged transcriptional regulation of SGK and ENaC: a link between hyperglycaemia and diabetic nephropathy

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Background and Aims: Alterations in the resorptive capacity of renal epithelia to sodium can have severe implications for the normal functioning of the nephron and are most likely to be prominent in the development and pathogenesis of secondary hypertension, a condition associated with a number of renal diseases including diabetic nephropathy and glomerulonephritis. One of the major regulators of sodium reabsorption in the nephron is the serum and glucocorticoid induced kinase (SGK), an aldosterone regulated gene, which mediates sodium reabsorption via its actions on the epithelial sodium channel (ENaC). Recent reports have identified SGK as a key signaling element whose level of expression appears to be upregulated in kidneys of diabetic humans and diabetic nephropathy. Hyperglycamia is believed to be the key pathophysiological component in promoting intercellular changes ultimately leading to the deranged transcriptional regulation of SGK.
Materials and Methods: RT-PCR, immunocytochemistry and western blot analysis confirmed that human cortical collecting duct (HCD) cells express SGK and alpha ENaC. For glucose, experiments, HCD cells were treated with (25 mM) glucose for 24 and 48 hours. For TGF-beta (2 nm) and Ionomycin (1 μm) experiments, HCD cells were treated for 4,6,8,12 and 24 hrs. A functional correlate for these increased levels of expression was provided by sodium microfluorimetry.
Results: Incubation of HCD cells with high glucose (25 mM) for 24 and 48 hours increased expression of SGK and Alpha ENaC protein (SGK, 261.7±14.9% of control (5 mM) at 48 hours; n=3, p<0.01%; alpha ENaC, 316.5%±9.9% of control (5 mM) at 48 hours n=6, p<0.01). Hyperglycaemia has been linked to both raised calcium and TGF-beta levels. Application of TGF-beta or Ionomycin alone induced a significant increase in SGK protein expression (TGF-beta, 125.5%±7.5% of control at 8 hours; n=3 p<0.001%; Ionomycin 263.7%±8.6% of control at 8 hours; n=3 p<0.001) suggesting a possible role for both TGF-beta and calcium in mediating downstream effects of hyperglycaemia. Intracellular sodium levels were found to be significantly elevated following 24 (107%±0.88%) and 48 (114%±0.93%) hours exposure to 25 mM glucose, as compared to cells cultured in low (5 mM) glucose.
Conclusion: This data taken in conjunction with that described in previous literature suggests that increases in intracellular sodium in response to high glucose after 48 hours may be mediated via the increased expression of both SGK and alpha-ENaC. This data highlights a possible link between hyperglycaemia and the deranged sodium reabsorption observed in cases of diabetic nephropathy, and may lead to identification of potential therapeutic targets.
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Renal oxidative stress in normotensive and hypertensive model of early experimental diabetes mellitus

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Background and Aims: Oxidative stress plays important role in the pathogenesis of nephropathy resulting from both diabetes and hypertension. These last 2 conditions frequently coexist in humans, and individually both of them can induce oxidative stress in the kidney. Recently oxidative stress was demonstrated in the renal cortex in hypertensive, but not in normotensive, model of early experimental diabetes. This finding prompted us to search for the mechanism of renal protection against oxidative stress in normotensive model, and the mechanism of renal oxidative stress in hypertensive model of early experimental diabetes mellitus.
Materials and Methods: Diabetes (D) was induced in spontaneously hypertensive rats (SHR) and their genetically normotensive control Wistar Kyoto (WKY) rats by streptozotocin at 12 weeks of age. After 10 days, rats were sacrificed and kidneys were collected. Renal cortical nitrotyrosine, receptor for advanced glycation end products (RAGE), and nicotinamide adenine dinucletide phosphate (NADPH) oxidase isoform Nox4, and p47phox subunit were assessed by Western blot. 8-hydroxy-2′-deoxyguanosine (8-OHdG) positive cells were evaluated by immunohistochemistry. Superoxide generation was assessed by lucigenin-enhanced chemiluminescence, and reduced glutathione (GSH) levels were quantified by chemical method.
Results: Plasma glucose levels were similar in WKY-D and SHR-D groups. Systolic blood pressure was higher (p<0.001, n=10) in control (C) and diabetic SHR than the WKY groups. Nitrotyrosine and 8-OHdG, markers of oxidative stress-induced protein and DNA modification, respectively, were found similar in the kidney in WKY-C and WKY-D, but they were elevated (p=0.068 in case of nitrotyrosine, p=0.016 in case of 8-OHdG; n=5) in SHR-D as compared with SHR-C. However, RAGE, Nox4 or p47phox levels were found similar between control and diabetic groups of both WKY and SHR strains. Non-enzymatic antioxidant GSH level was slightly increased in \WKY-D (4.60±0.33 μM/g tissue) than WKY-C (4.34± 0.36 μM/g), but it was significantly decreased (p=0.033, n=10) in SHR-D (3.70±0.25 μM/g) than SHR-C (4.20± 0.26 μM/g). On the other hand, we have observed an elevation of NADPH-induced superoxide generation in the renal cortex in both WKY-D and SHR-D groups as

compared with their respective controls in our initial experiments.

Conclusion: We conclude that the kidney of normotensive model, but not of hypertensive model, is apparently protected from oxidative damage in the very early stage of experimental diabetes. Although the significant depletion of the antioxidant GSH level in the kidney occurs only in hypertensive model, the pro-oxidant superoxide generation in the renal cortex appears to be elevated in both normotensive and hypertensive model of early experimental diabetes mellitus. Taken together, our data suggests that the impaired antioxidant defence mechanism may be responsible for the early susceptibility to renal oxidative stress in hypertensive model of diabetes. Currently we are evaluating enzymatic antioxidant status, and searching for the mechanism and source of superoxide generation in normotensive and hypertensive model of early experimental diabetes.

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Possible involvement of a VEGF receptor subtype-dependent mechanism in the pathogenesis of diabetic nephropathy in β cell-specific calmodulin overexpressing mice

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Background and Aims: Although diabetic nephropathy (DN) is a serious and life-threatening complication, there are very few proper animal models with distinct glomerular lesions mimicking human DN. We here characterized kidney lesions in β cell-specific calmodulin over-expressing (CaMTg) mice which experienced early-onset, marked and long-lasting hyperglycemia. Possible mechanisms underlying its pathogenesis were also investigated.

Materials and Methods: CaMTg mice were prepared using a transgene where the calmodulin gene was placed downstream the insulin promoter leading to pancreatic β cell-specific calmodulin overexpression in the ICR strain. CaMTg mice and non Tg mice were sacrificed at 3, 6 and 9 month-old (M) age. Renal functions of these animals were evaluated biochemically and histologically. Expression levels of type III, type IV collagens and α smooth muscle actin (αSMA) were examined immunohistochemically, and those of vascular endothelial growth factor (VEGF), VEGF receptor 1 and 2 (VEGFR-1, 2), and endothelial nitric oxide synthase (eNOS) were detected by Western blot analysis.

Results: CaMTg mice showed consistent and evident hyperglycemia, and increases of urinary protein excretion of at all the ages examined. Morphometrical analysis resulted in considerable increases of the glomerular and mesangial areas with Type III and IV collagen depositions as well as accumulation of αSMA in the CaMTg kidneys. Nodular lesions were seen in CaMTg mice at 6M and 9M, and even exudative lesions occasionally appeared in CaMTg mice at 9M. Vascular pole hyalinosis was also observed at 6M and 9M. In immunoblot analysis, expression levels of eNOS were decreased and those of VEGF were elevated in the 6M CaM Tg kidney. VEGFR-1 was less expressed in the 6M CaM kidney than nonTg one. Such difference between the CaM Tg and non Tg kidneys were not seen for VEGFR-2.

Conclusion: CaMTg mice developed multiple distinct lesions of the hallmark of human DN; i.e. nodular lesions, exudative lesions and vascular pole hyalinosis. Elevated VEGF levels and diminished eNOS expression may result in preferential activation of VEGFR-2, which is considered to cause endotherial cell proliferation. We expect this model is very useful in the elucidation of the pathogenesis of DN and in the development of its treatment.

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Retinopathy

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Effect of the oral protein kinase C β inhibitor ruboxistaurin on visual acuity in the PKC-DRS2 study

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Background and Aims: Patients with advanced non-proliferative diabetic retinopathy (NPDR) are at increased risk of vision loss. In the PKC-DRS2 study, orally-administered ruboxistaurin (RBX) mesylate, a protein

kinase C (PKC) β inhibitor, reduced the occurrence of sustained moderate visual loss (SMVL: ≥15 ETDRS letter loss sustained over the last 6 months of study participation) by 40% compared to placebo (from 9.1% to 5.5% of patients, p=0.034). The PKC-DRS2 also evaluated the effect of RBX 32 mg/day on categorical measures of visual acuity (VA).

Materials and Methods: The PKC-DRS2 study was a 36-month, randomized, double-masked, parallel, placebo-controlled, phase 3 study of 685 patients in 70 centers. Patients (placebo, n=340; RBX 32 mg/d, n=345) had moderately severe to very severe NPDR (ETDRS retinopathy score ≥47A and ≤53E), no prior panretinal photocoagulation, and a best-corrected VA of ≥45 letters (~6/38, 0.16 Snellen) in a study eye.

Results: VA of RBX eyes was better than that of placebo eyes after 12 months of therapy. The mean change in VA from baseline-to-endpoint (LOCF) was −0.8 letters, RBX, vs. −2.6 letters, placebo (p=0.014). During the study, 4.9% of RBX eyes gained 15 letters in VA, compared to 2.4% of placebo eyes (p=0.027). In addition to its positive effect on SMVL, RBX reduced sustained VA loss of 10 letters (30% reduction, p=0.043) and 5 letters (23% reduction, p=0.033) compared to placebo. There was a positive effect of RBX on VA loss both in eyes that did and did not receive focal photocoagulation during the study.

Conclusion: Compared to placebo, RBX reduced sustained 5-, 10-, and 15-letter loss in visual acuity and improved visual acuity more frequently over 36 months of treatment.

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Clinical safety of the selective PKC β inhibitor, ruboxistaurin, in patients with diabetic microvascular complications

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Background and Aims: Ruboxistaurin (RBX;LY333531) mesylate is a selective protein kinase C β (PKC β) inhibitor that has been demonstrated to reduce the risk of vision loss in patients with moderately severe to severe nonproliferative diabetic retinopathy. In preclinical animal studies, RBX at high and non-isoform-selective doses has been reported to be associated with reversible immune system dysfunction, cataractous lens changes, and hepatobiliary inflammation. We report here the safety profile from patients with diabetic complications treated with RBX (32 mg per day) for up to 3 years, which is critical information for the consideration of RBX as a treatment for diabetic retinopathy.

Materials and Methods: Data from patients with diabetes (1396 RBX; 1408 placebo [PL]) were combined from 11 Phase 2 and 3 placebo-controlled, double-masked studies. The patients enrolled had HbA_{1c}≤13%, blood pressure<190/105 and were diagnosed with at least one of the following diabetic complications: retinopathy, neuropathy, nephropathy, erectile dysfunction or peripheral vascular disease.

Results: Patients who were enrolled in the studies were predominantly caucasian (79%), males (70%), had type 2 diabetes (77%), a mean patient age of 54.7 years and a mean duration of diabetes of 14.8 years. Twenty three point two percent (327) of the PL-treated patients experienced ≥1 serious adverse event versus 20.8% (290) of the RBX-treated patients. There were 51 deaths (21 RBX; 30 PL) reported; none of the deaths was attributed to study drug by the investigators. The common (≥1/100 to<1/10) reported adverse drug reactions in the RBX treated patients were dyspepsia and increased blood creatine phosphokinase (CPK). Uncommon (≥1/1000 to ≤1/100) reported adverse drug reactions included atrioventricular first degree block (0.6%RBX; 0.1%PL), thrombosis (0.4%RBX; 0%PL), chalazion (0.4%RBX; 0%PL), posterior capsule opacification (0.5% RBX; 0.1% PL), micturition urgency, (0.4% RBX; 0%PL), and skin discolouration (0.5%RBX; 0.1% PL). Specific analyses based on the animal toxicology findings were conducted. No increased incidence of hepatobiliary disease, bacterial infections and cataracts (AREDs Lens Grading) was observed in the RBX group. RBX had no effect on glycemic control, blood pressure, incidence of severe hypoglycemia, or incidence of diabetic ketoacidosis. Incidence of patients reporting serious cardiac disorders was similar between the PL- (8.6%) and RBX-(7.6%) groups. No clinically relevant effects were observed on serial ECGs.

Conclusion: In controlled randomized clinical trials, RBX, a selective inhibitor of the PKC β isoform, did not alter glycemic control, had an AE profile comparable to PL and was well tolerated. RBX is the first member of a new class of therapeutics for the treatment of diabetic retinopathy.

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Automated identification of change in retinal images

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Introduction: Annual screening for retinopathy is recommended for diabetic patients. The optimal way is to use digital imaging after pupil dilatation. These images can easily be stored, retrieved and catalogued. However, in the UK about 2 million images annually have to be analysed. The aim was to create an automated software solution which could establish no-change criteria in images and allow only grading of retinal images where change of retinopathy had occurred.
Methods: We developed a computer system that could perform registration of retinal images after automatically adjusting to rotational, magnification, astigmatic, clouding and colour changes. Differencing was performed to allow the differences to become apparent. The change was then enhanced. A set of 30 randomly selected retinal image pairs was used for validation.
Results: The software adjusted to all changes, registered and differenced the image pairs in a mean time of 23.5 seconds with a success rate of 93%. The identification of common features in images had a success rate of 100%. Shadowing was found to be the cause of all misalignments.
Conclusion: The automated software allowed longitudinal changes to become apparent to the users and could detect the direction of the change (regression, progression). Additionally it could demonstrate where no change had occurred.

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Use of broadband technology and remote diagnosis of diabetic retinopathy

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Background and Aims: Transmission and receiving of high-resolution images has become easy with the widespread adoption of IT technology and broadband. As a result, remote diagnosis of images has been attempted in the medical field. It would be ideal for all patients with diabetes mellitus to periodically undergo ophthalmological examination, but this is difficult in reality, especially in remote areas where transportation and access to a specialist is limited. However, patients without symptoms may be more motivated to undergo examination in their primary care office. The other appeal is that findings can be displayed to the patient directly.
Retinal images were digitalized and a network filing system was introduced at our clinic. A remote diagnosis support system for the retinal images was also established to allow for the use of the broadband service. After repeated clinical trials an effective system was established.
Materials and Methods: Among the patients who underwent retinal imaging at our clinic, radiographs were interpreted for 106 patients who mainly suffered from diabetes mellitus. Retinal images were taken by a nurse using an EOS-D60 digital camera mounted on the CR6-45NM non-mydriatic retinal camera. The images were stored in a computer as DICOM files for real-time transmission to the computers of physicians. Current HbA1c and B.P etc. were recorded in the comments. The images were then transmitted via e-mail together with a referral letter. These files were encrypted for transmission to the ophthalmologist via a secured mail server made by Canon. The ophthalmologist interpreted the images after opening the attached file, recorded his/her findings on a form designed for this purpose, and sent the form back to our clinic.
Results: This system facilitated processes such as taking retinal images, transmitting them, and receiving reports. The images were recorded as 2048*1360 pixel full color JPEG/DICOM files and were rapidly transmitted via CATV broadband. The return e-mail from the ophthalmologist contained the assessment and the classification of retinal findings. The ophthalmologists determined that consultation had been necessary or desirable for 45% of the patients referred from our clinic. The ophthalmologist also commented that the 2048*1360 pixel images were of good quality for interpretation, and the functions such as zooming or altering brightness and contrast were useful for evaluation.
Conclusion: This system made it possible to accurately assess the changes of the retina in the asymptomatic diabetic patient. Concerns raised by non-specialist about abnormal findings being overlooked ware alleviated. In addition, it became possible to provide an accurate explanation with pictures of the findings to each patient. Ophthalmologist provided interpretations of the images, which was very informative for the physicians and expanded their knowledge. In conclusion, this system is useful because it can avoid the tragedy of progressive undiagnosed diabetic retinopathy in a patient with diabetes even though he/she is regularly seeing her primary care doctor. This system is also considered worth reporting because it can be introduced in any country and makes consultation with an ophthalmology specialist available even for patients living in remote areas or with limited health care facilities.

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Prevalence of retinopathy and eye lesions in a regional pilot project to monitor diabetes related complications 2002-2004 - the "Diabetes-TÜV" of the Deutsche BKK

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Background and Aims: In population based studies up to 30% of patients with diabetes manifestation already have diabetic retinopathy (DR). The prevalence of DR in Germany of all diabetic patients insured in a public health insurance (Deutsche BKK) was examined in the context of a general diabetes check-up ("Diabetes-TÜV") in a regional pilot project in one German Federal State (Lower-Saxony) from 2002 to 2004.
Materials and Methods: Patients of 351 primary care physicians were screened by ophthalmologists. The prevalence of diabetes related eye diseases documentated according to the screening sheet developed by the initiative group early diagnosis of diabetic eye diseases (Prof. Kroll, Marburg) was assessed. 6500 screening sheets were analysed (mean age 65±12,3 y, 48.5% female, diabetes duration 10.2±9.0J, 10,3% Diabetes Type 1). 14.5% multiple documentations occured.
Results: 84.5% of the patients had no DR. The mean diabetes duration was 9.8 years. In 4.4% of the patients a retina examination was impossible due to impaired visibility of internal eye structures. 11.2% of the patients had DR, mostly early stages (DR/diabetes duration: mild DR 8.8%/15.6 y.; severe non-proliferative DR: 1.8%/18,y.; proliferative DR: 0.6%/17.6 y.). A rubeosis iridis had 0.1%, macular oedema 0.9%, vitreous haemorrhage 0.3%, retinal detachment with or without macular lesion 0.1%, cataract or posterior capsular opacification 25.7%, an artificial lens 8.3%. Diagnostic and therapeutic actions recommended were fluorescent angiography 0.6%, a focal laser coagulation 0.1%, a panretinal laser coagulation 1.3%, a cryocoagulation 0.1% and vitrectomy 0.2%.
Conclusion: Documentation and completeness of the eye examinations in the "Diabetes-TÜV" of the Deutsche BKK were excellent. The prevalence of DR, even after long diabetes duration, is low compared with earlier population based studies in the USA (Wisconsin study) or Great-Britain (UKPDS) and slightly lower than published in Germany before. The better outcome could be due to improved diabetes care in Germany.

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Incidence of retinopathy in a cohort of type 2 diabetics during 10-year follow-up

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Background and Aims: Diabetic retinopathy remains the main cause of blindness in developed countries in spite of marked progress in the treatment of diabetes mellitus and wide use of lasertherapy. This fact points to the significance of many other circumstances. Therefore the undertaken study was aimed at a determination of incidence of diabetic retinopathy in a cohort of type 2 diabetics during a 10-year follow-up and to a delineation of the selected risk factors (age, sex, diabetes duration, BMI, fasting and postprandial glycemia, systolic and diastolic pressure, daily proteinuria, cholesterol, triglycerides, creatinine levels and insulintherapy). This could improve the pertinent prevention programs.
Materials and Methods: The study involved 1334 patients from an out-patient diabetological clinic. Diabetic retinopathy was diagnosed on the basis of ophthalmoscopy. Persons with diabetic retinopathy at the baseline examination were excluded from analysis of incidence. The cohort under study was submitted to the continuous programmed clinical observation.
Results: At baseline diabetic retinopathy was present in 419 persons (31.4%). During the 10-year follow-up diabetic retinopathy was found in 230 patients (25.7%) from a group of 915 patients without retinopathy at baseline. Therefore the cumulative incidence of retinopathy in the whole cohort was 49.0%. The incidence of retinopathy during follow-up was 38.9 per 1000 person-years. Mean age at the moment of diagnosis of new retinopathy was 63.8±9.7 years and mean diabetes mellitus duration was 12.0±5.5 years. 17 cases of proliferative retinopathy were diagnosed at the baseline, 35 additional cases developed during the 10-year follow-up. In the majority (94.3%) of patients with proliferative retinopathy diagnosed during follow-up, background retinopathy was present at baseline. The cumulative incidence of proliferative retinopathy in the whole cohort was 3.9%. Fasting and postprandial glycemia were higher in persons with retinopathy diagnosed during follow-up in comparison with those without any retinopathy. The former were characterized by longer duration of diabetes mellitus. A multifactorial regression analysis of Cox found that the predictors of retinopathy were: duration of diabetes and fasting glycemia. The statistical significance of several other risk factors could not be confirmed. During the 10-year follow-up 6 new cases of blindness occurred. Diabetic retinopathy was responsible for 5 of them (4 cases of proliferative retinopathy, 1 case with maculopathy).

Conclusion: During 10-year observation the newly diagnosed diabetic retinopathy developed in 25.7% of type 2 diabetics; the cumulative incidence reaches the level 49.0%. This is firmly connected as shown by multifactorial analysis to the diabetes mellitus duration and fasting glycemia. The significance of other factors could not be confirmed statistically in the studied cohort. The relation between the statistical and pathophysiological assessments is therefore also discussed.

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Optical coherence tomography findings in patients with diabetes mellitus type 1, with no or minimal diabetic retinopathy

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Background and Aims: Optical Coherence Tomography (OCT) is a non-invasive, non-contact, high resolution imaging technique, that acquires scans of the retina, providing information on morphology and pathological changes in the retina. OCT has enabled ophthalmologists to reliably detect and measure small changes in retinal thickness (RT), which can be presented in a topographic map. This allows for quantitative evaluation of RT in patients and monitoring of the efficacy of different therapies. The topographic maps are obtained from 6 radial, cross-sectional scans centered on the fovea. RT is calculated automatically by the OCT analysis software in macular areas centered around the fovea. Recent studies in mixed type 1 and type 2 diabetes mellitus (DM) populations suggest that the retina is thickened, even in a preclinical phase of diabetic retinopathy (DRP) and especially in a circular area directly surrounding the fovea. The first aim of this study is to evaluate the RT measured with OCT exclusively in patients with DM type 1, with no or minimal DRP, and compare this to a healthy control group. Secondly, we evaluated the possible association of metabolic control and RT.
Materials and Methods: Patients with DM type 1, with minimal or no DRP on biomicroscopy and stereo fundus photography, seen at the outpatient clinic of the department of internal medicine at the AMC, were included and underwent a full ophthalmologic examination, fundus photography and OCT scanning of the macula. Mean RT measurements were taken in the pericentral area around the fovea (donut shaped ring with inner diameter of 1 mm and outer diameter of 3 mm) and the peripheral area around the fovea (inner diameter of 3 mm and outer diameter of 6 mm). RT measurements in patients with minimal DRP were compared to RT measurements in patients without DRP and age and gender matched healthy subjects. Parameters of metabolic control (blood pressure, HbA1c, cholesterol, triglycerides, TSH, creatinine clearance) were recorded simultaneously and correlated to the RT measurements.
Results: Fifty-two patients were included in the study. 23 patients had minimal DRP and 29 showed no signs of DRP. Mean RT in the pericentral area was 268 μm (±19 μm) in patients with minimal DRP, 278 μm (±16 μm) in patients without DRP compared to 281 μm (±13 μm) in healthy subjects (n=29). The difference in pericentral RT between the patients with minimal DRP compared to the controls was statistically significant (p-value=0.008). No significant difference was found between the groups for the RT in the peripheral area. No correlation was found between RT measurements in the patients and any of the metabolic parameters.
Conclusion: In contrast to earlier reports we could not demonstrate an increased RT of the pericentral or peripheral area in patients with DM type 1 with no or minimal DRP. Instead, we found a decreased pericentral RT in patients with minimal DRP compared to healthy controls. This could be explained by a loss of intraretinal neurons in the earliest stage of DRP. The potential role of loss of retinal thickness as an early marker of incipient diabetic retinopathy deserves prospective study.

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The effect of activated inflammatory cells in diabetic retinopathy

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Background and Aims: Some studies postulated that inflammation might play an important role in the development of Diabetic Retinopathy (DR). It was stated that monocytes/macrophages were involved in the initiation process of microvascular pathology characteristics of DR. In addition, several studies have reported that diabetic leukocytes are activated shown by increased expression of adhesion molecules and by greater adhesion to endothelial cells. Furthermore, up regulation of adhesion molecules are confirmed in leukocytes of DR patients.
The aim was to study the expression of adhesion, activation, chemokine receptors molecules, to measure the

cytokines levels and to correlate the type of expression with the adhesiveness of leukocytes to retinal vessels *in vivo*.
Materials and Methods: Diabetes was induced in mice by intraperitoneal injection of streptozotocin (STZ) and mice were analysed after 2 weeks. Immunofluorescence staining was performed in fixed whole flat mount retina and stained leukocytes were counted using a fluorescence microscope and recorded images were taken under a confocal laser scanning microscope. Myeloid bone marrow and leukocyte blood cells of wild-type and diabetic-induced animals were analysed by flow cytometry to quantify monocyte (CD11b), activation cell surface (CD40) and chemokine receptor (CCR5) markers. Cytometric bead assay and ELISA methods were realized in blood serum and supernatant of monocytes culture cells of these mice in order to measure the cytokine secretion. The data were expressed as mean±SD. Statistical significance was determined by using a One-Way Analysis of Variance followed by Bonferroni's Multiple Comparision test and Paired Student T-test.
Results: Identification and statistically significant increased numbers of adherent CD11b cells were localized inside of the endothelial capillaries of diabetic retinea. The flow cytometric analyses resulted in a significant enhanced of CD40 and CCR5 expression on slightly increased number of diabetic myeloid bone marrow cells. Unchanged number of leukocyte blood cells shown a tendency to increased expression of CD40 in diabetic comparing to wild type animals.
Conclusion: The STZ induced-diabetic mice presented adhesive and activated myeloid cells inside of the retinal vessels. In summary, the data suggest that diabetes-induced leukocytes capillary occlusion in Diabetic Retinopathy.
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Inflammation but not oxidative stress gets elevated in the retina in a model that combines genetic hypertension and experimental diabetes

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Background and Aims: Hypertension is the main secondary factor associated with diabetic retinopathy (DR). The mechanisms by which hypertension increase the risk for DR are poorly understood. Recent data have revealed that oxidative stress and inflammatory responses are involved in pathogenesis of DR activating the transcription factor nuclear kappa beta (NF-kB). But the crosstalk between the redox status, inflammation and RAGE (AGE-receptor) system in diabetic retinopathy is not understood. The aims of this study were to investigate the role of inflammation and oxidative stress in experimental model that combines hypertension and diabetes.
Materials and Methods: Four week old spontaneously hypertensive rats (SHR) and their normotensive counterpart Wystar Kyoto (WKY) rats were rendered diabetic by intravenous injection of streptozotocin. After 20 days, rats were sacrificed and retinas were collected.
Results:The number of immunohistochemically detected ED1/microglial positive cells and the expression of ICAM-1 in the retina were significanthy higher in diabetic SHR than in the control ($p<0.0001$). The levels of reduced glutathione were lower in diabetic SHR in comparison with WKY groups ($p<0.04$) but the NADPH oxidase, evaluated by expression of NOX-4 subunit, did not differ in groups. NF-kB p65 levels were higher in SHR compared with WKY groups ($p<0.005$).
Conclusion: The concomitance of diabetes and hypertension leads to exuberant inflammatory response but not oxidative stress in the retina. Perhaps the RAGE system can be involved in the activation of NF-kB. This system and its blockage are under investigation.
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Diabetes mellitus and the eye: axial length

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Background and Aims: The refractive error of the eye is dependent on its axial length. Refractive error is known to fluctuate significantly in poorly-controlled diabetic patients. Recently it has been reported that human eyes fluctuate in axial length during the day. However, this change is not detectable in all subjects, suggesting physiological influences such as diet. The purpose of this study was to investigate fluctuations in axial length and blood glucose levels (BGLs) in diabetic patients and control subjects, using partial coherence interferometry.
Material and Methods: Periodic axial length measurements were taken with an IOLMaster (Zeiss, Oberkochen, Germany) in 21 type 2 diabetic subjects (age 56±11 years), 19 type 1 diabetic subjects (age 38±15 years) and 18 non-diabetic controls (age 46±22 years). Additionally, BGLs were measured using a HemoCue Beta glucose analyser (HemoCue, Ängelholm, Sweden). Measurements were taken between 8AM and 8PM at approximately two hourly intervals. The ocular length variability was mapped against

time of day and related to BGLs, age, and the degree of diabetic retinopathy.

Results: Axial length variations were found to be significant with time (ANOVA, P=.003) and there was a significant effect for the interaction between group and time of day (ANOVA, P<0.0005). DM type 1 and control subjects showed a significantly increased axial length in the afternoon compared to in the morning (t-test, P<0.0005). No significant change was observed among DM type 2 subjects. Axial length fluctuated sinusoidally with time of day. A three-parameter sinusoidal curve was used to fit each set of axial length measurements, which resulted in a correlation coefficient $R^2 \geq 0.7$ in 62% of type 2 diabetic subjects, 78% of type 1 diabetic subjects and 72% of control subjects. Multiple regression analysis established that neither BGLs, age, nor the degree of diabetic retinopathy had a significant effect on the fluctuations of the axial length (P>.05)

Conclusion: Axial length varies throughout the day in diabetic and control subjects; however, this change does not appear to be significant in every individual. The data suggest a maximum axial length value in the afternoon. Changes in axial length from baseline did not correlate with BGLs, age, or the degree of diabetic retinopathy. Therefore, blood glucose levels may not be the main physiological influence in axial length fluctuations.

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PS 95

Somatic neuropathy

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RAGE-dependent impairment of glyoxalase-1 contributes to functional deficits in diabetic neuropathy

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Background and Aims: The Glyoxalase I (GO1)-system is a central part of the cellular defense detoxifying precursors of Advanced Glycation Endproducts (AGEs) such as methylglyoxal, glyoxal and other alpha-oxoaldehydes. Since AGEs, binding of AGEs to the receptor RAGE and subsequent RAGE dependent cellular activation are supposed to contribute to the development and progression of late diabetic complications we studied the interaction of RAGE and GO1 *in vitro* and in experimental animal models of diabetic neuropathy (DN).

Materials and Methods: GO1-transcription (Real time PCR), -expression (Western Blot) and -activity (enzyme assay) and its modulation by RAGE-ligands, determined in transient transfection studies using murine GO1-promoter plasmids, was measured in RAGE bearing (WT) and RAGE deficient ($RAGE^{-/-}$) fibroblasts, endothelial cells and dorsal root ganglia (DRG). In vitro studies were paralleled *in vivo* by immunhistochemistry of sciatic nerves and experimental models of DN including, pain perception assays and determination of neuronal blood flow in healthy and diabetic WT- and $RAGE^{-/-}$ - mice.

Results: Methylglyoxal and AGEs, but also other RAGE-ligands such as S100B significantly reduced GO1-transcription, expression and activity in WT derived fibroblasts, endothelial cells and dorsal root ganglia cells (DRG). In $RAGE^{-/-}$cells, however, RAGE ligands induced a significant upregulation of GO1 implying that engagement of RAGE directly suppressed GO1. Consistently, GO1-expression was increased in WT cells treated with the RAGE antagonist sRAGE (25 μg/ml) for 48 h. To study a potential role of RAGE dependent GO1-reduction in DN, GO1-transcription and expression was determined in sciatic nerves from healthy and diabetic WT and $RAGE^{-/-}$mice. Compared to $RAGE^{-/-}$mice, GO1 was significantly lower in peripheral nerves of WT-mice and further attenuated in diabetes. The functional significance of GO1 deficiency for DN was studied in diabetic mice, kept in the diabetic state for 4 months in the absence or presence of an inhibitor of GO1. GO1-inhibitor treatment resulted in significant increased pain perception in early stages of DN and in impaired neuronal blood flow (NBF) in both, diabetic WT and $RAGE^{-/-}$mice, whereas impairment was even more pronounced in $RAGE^{-/-}$mice.

Conclusion: These data provide firm evidence that the diabetes dependent reduction in GO1, which contributes to functional deficits in DN, is at least in part caused by RAGE-ligand interaction.

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Metabolic syndrome in type 1 diabetes: a potential risk factor for the development of diabetic neuropathy after 9 years

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Background and Aims: Cardiovascular risk factors such as hypertension, lipids, smoking have been well recognised as a risk factor of diabetic neuropathy (DN). Metabolic syndrome (MS) is one of the important cardiovascular risk factor and can be present even in type 1 diabetes. However, the association of MS to the development of DN is still not clear. In Sheffield Prospective Diabetes Study we investigated if the presence of MS predicts the future development of DN. Our aim was to follow up a well-defined cohort of subjects with newly diagnosed type 1 diabetes and to investigate the relationship between the presence of MS to the development of DN.
Materials and Methods: 66 newly diagnosed type 1 diabetic subjects (mean age 31+/− 9(SD) duration (3 years +/− 2) were identified and followed for 9 years. They had detailed neurological assessment (symptoms and signs score, nerve conduction, vibration perception threshold, warm thermal discrimination threshold and autonomic function tests) and blood tests performed at base line, 3 years and 9 years. Metabolic syndrome was defined using NCEP Adult Treatment Panel III criteria. DN was defined as per Dyck's criteria
Results: At the 9 years, 51 patients were followed up and there were no difference between these subjects and those who were lost to follow up.Overall presence of MS was 21.2% at baseline which rose to 31.4% by 9 years follow up. In subjects with MS, DN was present in 60% in compariosn to 29.3% in subjects without MS (p=0.03). 9 subjects already had DN at baseline and were excluded from further analysis. Of the remaining 42 subjects 33.3% subjects with MS at baseline developed new onset DN, whereas only 19.4% without MS developed DN (p=0.001).
Conclusion: This prospective study suggests that as other cardiovascular risk factors, MS may also be an important risk factor for the development of DN. Aggressive management of the risk factors of MS is essential to reduces cardiovascular mortality and possoibly morbidity due to DN.
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Metabolic syndrome includes augmented, not impaired, insulin-mediated vasodilation of the cutaneous microcirculation

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Background and Aims: Insulin is reported to improve capillary flow, increasing both arteriolar diameters and erythrocyte flow velocity. However, hyperinsulinemia may also exert a negative effect on erythrocyte deformability, thus impeding flow. While capillary perfusion is thought to be impaired among diabetic subjects, we have previously described that thermally- induced hyperemia in the skin microcirculation, based on percent increase in perfusion over baseline, was significantly greater among prediabetic compared to normal monkeys, and that this finding was significantly correlated with circulating levels of hsCRP. The study was conducted to better understand the interactions between insulin, the metabolic status and tissue perfusion.
Materials and Methods: Twenty normoglycemic (N) (FPG<110 mg/dl) non human primates, 8 of which were prediabetic with characteristics of the metabolic syndrome (PreDM/MetSyn), underwent the hyperinsulinemic, euglycemic clamp procedure. Microvascular flow was assessed using a laser Doppler flowmeter. Baseline and thermally-stimulated perfusion levels were measured before and at 10 minute intervals following insulin infusion for a period of 60 minutes. Immunohistological analyses using various neuronal markers were performed on skin biopsies.
Results: Mean basal perfusion improved over pre-clamp levels 60 minutes after insulin infusion (from 36.7±7.9 to 104.9±20.2 P.U., p=.0002) and heat- induced peak flow was also markedly enhanced during the hyperinsulinemic period (from 87.4±13.8 to 157.8±25.6 PU, p<0.0004) in all monkeys. The per cent increase in perfusion during heating, corresponding to microvascular recruitment evoked by the axon flare response, was significantly higher among PreDM/MetSyn subjects compared to normal similar aged monkeys. Extensive neurovascular remodeling, including proliferation of capillaries in the dermis and an increase in CGRP/SP - expressing C- fiber innervation to the epidermis during the prediabetic/metabolic syndrome phase of the disease were also observed.
Conclusion: The alterations in the cutaneous angioarchitecture and its innervation are likely to provide the morphological basis for the increased neurogenic vasodilation seen in prediabetes, a prodrome to the later deterioration in heat-stimulated and flow -mediated dilation seen in the more advanced stages of diabetes in humans and in monkeys. Thus augmented insulin-stimulated flow is likely to be a marker of progressing cutaneous pathology leading to the distal micorvascular complications of diabetes.
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Cardiovascular disease, diabetic polyneuropathy and ACE gene polymorphisms in the North Catalonia Diabetes Study (NCDS)

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Background and Aims: We observed several relationship between cardiovascular disease prevalence (CVD) and polymorphisms of Renin-Angiotensin axis gene regulation. The aim was to evaluate the possible association between diabetic polineuropathy (DPN) and ACE gen polymorphism (insertion/delection (I/D) in a random sample of diabetic population obtained from the North Catalonia Diabetes Study (NCDS).

Materials and Methods: Forty type 2 diabetic patients (T2DM) with CVD and thirty one without CVD (matched for gender an age) were selected. Previouly, they were selected for evaluation of non classical cardiovascular risk factors.

DPN was assessed by a clinical neurological evaluation (bilateral affectation of vibration perception thresholds with neurothesiometer and tuning fork, sensory tests to touch, pinprick and cold perception, Semmes-Weinstein Monofilament 5.07, muscle strength and reflexes according to the Michigan Diabetic Neuropathy Score and the items of modified Neuropathy Symptom Score). CVD includes stroke, coronary and peripheral ischemia

Results: Diabetic polyneuropathy (DNP) was related to cardiovascular disease (CVD) prevalence (p=0.036) in the NCDS Study. In the evaluation of T2DM with CVD, patients with DPN manifested less D/I heterozygous genotype than patients without DPN (p=0.012).

In logistic regression analysis using D/I-additive model, the D/I allele combination manifested some protective effect on diabetic polyneuropathy prevalence (odds ratio: 0.081; 95% confidence interval, 0.009–0.720). T2DM patients without CVD did not observe any significant difference only a tendency (p=0.08). In contrast, the distribution of other homocygous genotypes [D/D or I/I allele combination] did not differed in both T2DM patients with or without DPN.

Conclusion: Our results suggest that the heterozygous genotype, combination of D/I allele, could be a protector factor for DPN presence in this previous study of T2DM with CVD.

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The new indicator test (Neuropad) in the assessment of the staged severity of neuropathy

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Background and Aims: The new indicator test for sudomotor function (Neuropad®) has been shown to be a highly sensitive and reproducible tool for the diagnosis of diabetic peripheral neuropathy. The aim of this study was to examine the utility of the indicator test in the assessment of the staged severity of diabetic neuropathy.

Patients and Methods: This study included 120 type 2 diabetic patients (58 men) with a mean age of 67.3±5.9 years and a mean diabetes duration of 13.1±3.2 years. Neuropathy was diagnosed and staged by clinical examination and nerve conduction study, according to the Michigan classification system (Feldman et al, 1994). Patients were also examined with the indicator test, applied on the plantar aspect of the feet. Time until complete colour change of the test was recorded and stratified into deciles according to the spread of measurements in the study population.

Results: Neuropathy was staged as class 0 in 37 patients, class 1 in 44 patients, class 2 in 28 patients and class 3 in 11 patients. Time until complete colour change was 436.5± 62.9, 740±88.1, 1192.5±161 and 1817.3±127.4 seconds in patients staged as class 0, 1, 2 and 3 respectively (p=0.001). Use of a threshold lower than 530 seconds until complete colour change had 97.3% sensitivity and 100% specificity for diagnosis of class 0. Use of a threshold lower than 1000 seconds until complete colour change had 100% sensitivity and 97.4% specificity for class 1 neuropathy. A threshold lower than 1440 seconds had 92.9% sensitivity and 100% specificity for class 2 neuropathy. A threshold above 1440 seconds had 100% sensitivity and 99% specificity for class 3 neuropathy. A highly significant (Kendall's tau-b=0.848, p=0.001) correlation was shown between time until complete colour change of the test and Michigan class of neuropathy.

Conclusions: It appears that the indicator test contributes substantially to the assessment of the staged severity of neuropathy. There is excellent agreement between the indicator test and the Michigan classification system. These results suggest a role for the indicator test in the assessment of diabetic neuropathy.

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Frequence modulated electromagnetic neural stimulation enances cutaneous microvascular perfusion in patients with diabetic neuropathy

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Background and Aims: As a non pharmacological treatment, Frequency Modulated Electromagnetic Neural Stimulation (FREMS) has recently demonstrated, in a placebo-controlled trial, to be a safe and effective treatment of painful diabetic neuropathy, leading to amelioration of symptoms and improvement of some parameters of peripheral nerve function. We here present the results of an ancillary study of this trial, investigating the effects on microvascular function.
Materials and Methods: Patients (n=31) with painful neuropathy associated with decreased motor and/or sensory nerve conduction velocity (<40 m/s) in at least one nerve trunk of the lower limbs and increased vibration perception threshold (>25 V) were enrolled in a randomised, double-blind, crossover FREMS vs placebo study. Each patient received two series of ten treatments of either FREMS or placebo in random sequence, each lasting no more than 3 weeks. Measures at baseline, end of FREMS and placebo series and after 4 months of follow up included: cutaneous blood flow by laser doppler flowmetry, partial tissue tension of oxygen (TcpO2) and carbonic anhydride (TcpCO2) by oxymetry (Clark electrode) at the lower extremities in basal resting conditions and as incremental response after a thermal stimulation.
Results: Cross-over analysis showed no differences between FREMS and placebo as cutaneous blood flow and TcpCO2 and a significant reduction of TcpO2 after FREMS in resting conditions (p=0.007), but not as incremental flow after warming. At 4 months, a significant increase of resting cutaneous blood flow was observed (p=0.0033 vs baseline), with no differences as incremental flow after warming; no differences were observed for TcpO2 and TcpCO2 either in resting conditions or as response to thermal stimulation.
Conclusion: These findings indicate that ten treatments with FREMS induce an enhancement of microvascular blood flow measurable up to 4 months of follow-up, and a short term reduction of TcpO2, possibly due to increased epidermal oxygen consumption. The increased cutaneous blood flow could reflect an increased endoneural blood flow and this effect might contribute to explain the improvements of some parameters of peripheral nerve function induced by FREMS.

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Numbness in both toes and soles and disproportional sweating restricted on face and chest are reliable and useful symptoms for diagnosis of diabetic polyneuropathy

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Background and Aims: Though symptoms and signs of diabetic symmetric polyneuropathy (DPN) are well known experientially, the reports which examined the specific symptom of DPN based on multiple objective neurological tests are very rare. We aimed to reconfirm the specific symptoms and signs of DPN using 7 objective nerve function tests in 593 diabetic patients. Furthermore, the prevalence of the characteristic symptoms in Japanese type 2 diabetic outpatients under primary-care physicians was examined using a nationwide questionnaire survey.
Materials and Methods: *I. Specific symptoms and sign of DPN* Subjective symptoms: “numbness in toes and soles”, “pain in feet”, “numbness in hands”; “pain in hands”; “coldness in both feet”, “frequent leg cramp”, “orthostatic dizziness”, “sweating restricted on face and chest”, “frequent constipation/diarrhea” were investigated by interview. “Achilles tendon reflex (ATR)” and “grasping calf pain” were examined as neurological physical signs. The relationships between symptoms, signs and 7 objective nerve function tests such as: motor nerve, sensory nerve and F-wave conduction velocity, sensory nerve action potential, coefficient of variation of R-R intervals in electrocardiogram, fall in systolic blood pressure during head-up tilt and quantitative vibratory perception threshold were evaluated. *II. Prevalence of the neurological symptoms* The questionnaire survey which was a part of the nationwide survey focused on self-management of the diabetes and erectile dysfunction was performed on 1524 male diabetic outpatients of 119 hospitals or clinics. Additional survey was also carried out on 93 outpatients of the local public hospital (Shingu municipal medical center: SMC) to confirm the absence of gender difference in symptoms.
Results: *I. Specific symptoms and sign of DPN* ;1) bilateral not unilateral “numbness in toes and soles” and “diminished ATR” were significantly related to the all nerve functions, 2) bilateral “pain in feet”, “grasping calf pain” and “sweating restricted on face and chest” were significantly related to 6 nerve functions, 3) “numbness in hands”, “frequent leg cramp” and “coldness in feet” were related to only a few nerve function. *II. Prevalence of the neurological symptoms* ; There was no gender difference in all symptoms and signs in SMC survey, and the

prevalence of symptoms and signs were almost same between national and SMC surveys. The prevalence of bilateral "numbness in toes and soles", "pain in feet" and "sweating restricted on face and chest" were 25%, 10% and 15%, respectively.
Conclusion: Bilateral "numbness in toes and soles" and disproportional "sweating restricted on face and chest" seem to be reliable and useful symptoms for diagnosis of DPN. Bilateral "diminished ATR" and "grasping calf pain" also seem to be characteristic signs of DPN.

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A provisional evaluation of the validity of the modified clinical neuropathy examination (mCNE)

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Introduction: Diabetic neuropathy is a common and serious complication of diabetes. Various methods are used to assess the severity of neuropathy. In this study we aimed to evaluate the inter-observer repeatability and criterion validity of the mCNE.
Methods: Forty six diabetic patients, aged 32 to 70 years, 35% Type 1, mean duration of diabetes 16.7 years, were examined at baseline and followed up after ~1 week. Different methods of assessing neuropathy were used by 2 examiners. These were the mCNE (cut off for normality=>4=neuropathy), Vibration Perception Threshold (VPT), modified Neuropathy Disability Score (mNDS), Nerve Conduction Studies at right peroneal nerve, [(NCS) cut off for normality >42 m/s=neuropathy].
We analysed correlation between mCNE & VPT as well as NCS and NDS.
The inter-observer agreement between baseline and follow up was assessed using a kappa statistic. Sensitivity and specificity values were calculated comparing mCNE with NCV=>42 m/s.
Results: The sample population had different levels of neuropathy with a mean VPT 25±15.9 volts, mNDS 5 (IQR 1–8) and NCV of 41.2±6.1 m/s. mCNE correlated well with VPT [correlation coefficient (CC) 0.86]; NCV [CC: −0.76], NDS [CC: 0.92]. The inter-observer agreement was the same at baseline and and follow (kappa 0.91; 0.62,1.2). When compared with the gold standard NCS, the sensitivity of the mCNE was: 0.87; 95% CI (0.64,0.97) while specificity was: 0.93; 95% CI (0.72,0.98).
Conclusions: These results suggest that the modified Clinical Neuropathy Examination is a good method of assessment of diabetic peripheral neuropathy for research and clinical use.

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Decreased vibration perception threshold in type 1 but not type 2 patients with proliferative retinopathy

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Background and Aims: The aim of this study was to examine the prevalence of neuropathy and nephropathy in patients with type 1 (T1DM) and type 2 (T2DM) diabetes complicated by proliferative retinopathy (PDR).
Materials and Methods: Comparisons were made between 18 patients with T1DM and PDR and 19 age and diabetes duration matched controls without PDR, and also between 24 patients with T2DM with PDR and 20 diabetes duration matched controls. Clinical examination of the eyes was performed through dilated pupils using a slit lamp and a magnifying lens. Vibration perception threshold (VPT) was measured by semiquantitative tuning fork C128 (grade 0–8) and ankle reflexes were recorded. Body weight (Kg), serum creatinine, albumin, fibrinogen and urine albumin concentration were measured, and the presence of macrovascular (coronary, cerebrovascular and peripheral arterial) complications was also documented.
Results: VPT was significantly worse in patients T1DM and PDR compared with controls (4.7±1.9 vs. 7.3±0.73, $p<0.01$), and ankle reflex score was also worse (3.56±1.04 vs. 1.9±1.74, $p<0.01$). Vibration perception was negatively correlated with body weight ($r=-0.37$, $p=0.02$), proteinuria ($r=-0.52$, $p<0.01$), creatinine ($r=-0.50$, $p<0.01$), albuminaemia ($r=-0.41$, $p=0.01$), fibrinogen ($r=-0.39$, $p=0.02$). After multiple regression analysis the correlations with age ($p=0.02$), proteinuria ($p=0.01$) and creatinine ($p<0.01$) remained significant. In T2DM patients with PDR were younger than controls (60.7±8.6 vs. 67.5±6 years, $p<0.01$), but VPT was not significantly different between the two groups (5±2.8 vs. 6.1±1.6, $p=0.13$). VPT was negatively correlated with duration of diabetes ($r=-0.30$, $p=0.05$), duration of insulin therapy ($r=-0.33$, $p=0.03$) in T2DM. A positive history of macrovascular disease was more common in T2DM without DPR than among T2DM with PDR (20.8% vs. 55%, X^2 5.5, $p=0.02$; 33.3% vs. 70%, X^2 5.88, $p=0.02$).

Conclusion: Worsening VPT in T1DM is strongly associated with diabetic nephropathy and diabetic proliferative retinopathy. In T2DM it is possible that endogenous insulin may exert a protective effect on microvascular complications while also having an adverse effect on the incidence of macrovascular complications.

Thanks for suggestions to William Jeffcoate

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Somatic neuropathy - pathogenesis and treatment

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Activation of Na^+/H^+−exchanger-1: a novel pathogenetic mechanism in peripheral diabetic neuropathy (PDN)

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Background and Aims: Overall hypothesis: Enhanced Na^+/H^+−exchange (NHE) results in cytosol alkalinization and activation of the upper part of glycolysis which, under conditions of diabetes-associated inhibition or insufficient upregulation of glyceraldehyde 3-phosphate dehydrogenase, causes diversion of the glycolytic flux towards several pathways implicated in diabetic complications. The purpose of our study was to explore the role for Na^+/H^+-exchanger-1 (NHE-1), the most abundant NHE isoform, in PDN.
Material and Methods: In vivo experiments were performed in 1) control and STZ-diabetic rats treated with/without the NHE-1 inhibitor cariporide; 2) control and STZ-diabetic NHE-1+/+and+/− mice. In vitro studies were performed in human Schwann cells (HSC) cultured in 5 mM or 30 mM glucose. Nerve blood flow was measured by microelectrode polarography and hydrogen clearance, thermal algesia by paw withdrawal and tail-flick tests, mechanical algesia by von Frey anesthesiometer/rigid filament and Randall-Sellito tests, and tactile allodynia by flexible von Frey filament test. Oxidative stress was assessed by immunohistochemistry [nerve nitrotyrosine and poly(ADP-ribose)] and lucigenin test (aortic superoxide production). In vitro studies employed real-time PCR, Western blot analysis and BCECF test of NHE-1 activity.
Results: NHE-1 is abundantly expressed in rat and mouse sciatic nerves and DRG neurons and its expression is increased in diabetes. Nerve blood flow (microelectrode polarography/hydrogen clearance) and sensory nerve conduction velocity (SNCV) deficits were prevented, and motor nerve conduction velocity (MNCV) deficit, thermal hyperalgesia, mechanical hyperalgesia, tactile allodynia, nerve nitrotyrosine and poly(ADP-ribose) accumulation, and aortic superoxide production alleviated in STZ-diabetic rats treated with cariporide compared with untreated diabetic rats. MNCV deficit, thermal and mechanical hypoalgesia and tactile allodynia were less severe in NHE+/− than in NHE-1+/+STZ-diabetic mice. High glucose-induced NHE-1 mRNA and protein overexpression and NHE activation were clearly manifest in HSC, and were mediated via poly (ADP-ribose) polymerase-1 (PARP-1) and MAPK (ERK, SAPK/JNK)-involved mechanisms.
Conclusion: NHE-1 activation is a novel fundamental mechanism in the pathogenesis of PDN, and is likely to be implicated in neuropathy in human subjects with diabetes.

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Neuronal mitochondrial degeneration in diabetic rats and ameliorative effects of pioglitazone

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Background and Aims: Mitochondrial degeneration and induction of oxidative stress has been considered to play a role in the progression of diabetic complications. Peroxisome proliferator-activated receptor gamma coactivator-1α (PGC1α) is a co-activator that increases mitochondrial number and proteins by coactivating and inducing transcriptional factors such as Nrf-1 and Tfam. The aim of this study was to investigate 1) mitochondrial degeneration in neuronal tissues of diabetic animals, 2) the changes in PGC1α and its related factors, and 3) the effects of pioglitazone, a PPARγ agonist, in normalizing these changes.
Materials and Methods: Adult rats were made diabetic with 45 mg/kg streptozotocin (STZ). Pioglitazone (20 mg/kg) or vehicle (0.5% carboxymethylcellulose) was administered once daily by gastric gavage within one week of the onset of diabetes. For neuropathy measurement, tactile allodynia and nerve conduction were examined for 3 months after STZ injection. For tissue analysis, dorsal root ganglion (DRG) neurons were collected at 1 and 3 months. To determine the mitochondrial DNA copy numbers, total DNA was extracted and NADH dehydrogenase 1 (mitochondrial genome) was measured using a real-time PCR technique, which was then normalized against the amount of lipoprotein lipase (nuclear genome). For gene expression

analysis, PGC1α and related genes involved in mitochondrial homeostasis were measured using real-time PCR.
Results: Both motor and sensory nerve conductions were slowed at 1 and 3 months by diabetes. Tactile allodynia was seen only after 2 months of diabetes. Mitochondrial DNA was decreased at both 1 and 3 months (11 and 27% decrease respectively) and loss was greater in animals with more severe neuropathy. PGC1α was not changed at 1 month but increased at 3 months by 11%, a change that is associated with upregulation of Nrf1 and Tfam (23 and 26% increase respectively at 3 months). All changes were statistically significant. Pioglitazone treatment improved neuropathy and prevented the loss in mitochondrial DNA in diabetic animals ($P<0.05$). Pioglitazone also reduced gene expression changes in PGC1α and downstream transcriptional factors.
Conclusion: In DRG from chronic diabetic rodents, there is loss of mitochondrial DNA. In response, PGC1α, Nrf-1 and Tfam are upregulated. Pioglitazone prevents mitochondrial DNA loss and normalizes gene expression changes suggesting protection against mitochondrial degeneration under diabetic conditions.
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Effect of pioglitazone in experimental diabetic neuropathy

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Background and Aims: Generation of oxidative stress from mitochondria plays an important role in the progression of diabetic complications. Uncoupling proteins (UCPs) may be preventing excess amount of oxidative stress generation in mitochondria and recently was shown that its polymorphism is associated with human diabetic neuropathy. In dorsal root ganglion (DRG) of diabetic rats, UCP-3 proteins decrease suggesting the loss of antioxidative defense. It has been also shown that overexpression of UCPs protects DRG neurons from oxidative injury under high-glucose conditions. These factors regulating mitochondrial homeostasis may be controlled by key gene regulators that include Peroxisome Proliferator-Activated Receptor γ (PPARγ). The aim of this study was to investigate the neuroprotective effects and mechanisms of pioglitazone, a PPARγ agonist, in diabetic rats.
Materials and Methods: The study was designed with four animal experimental groups; 1) non-diabetic control, 2) non-diabetic+pioglitazone, 3) diabetic control, 4) diabetic +pioglitazone. Adult rats were made diabetic with 45 mg/kg streptozotocin. Pioglitazone (20 mg/kg) or vehicle (0.5% carboxymethylcellulose) was administered once daily by gastric gavage within one week of the onset of diabetes. For neuropathy measurement, tactile allodynia and nerve conduction were examined for 3 months after STZ injection. In tissue analysis, DRGs were collected at 3 months and gene expression was analyzed using real-time PCR technique.
Results: Tail motor nerve conduction was slowed by diabetes (42.2+/− 1.0 in non-diabetic control vs 39.0+/− 0.8 in diabetic control, m/s, $P<0.05$), which was recovered by pioglitazone treatment (42.7+/− 1.2 in diabetic +pioglitazone, $P<0.05$). Tactile allodynia was observed in diabetic group and it was improved by pioglitazone treatment. In diabetic animals, PPARγ expression was increased by 58%, UCP-2 and −3 was increased by 31% and 43% respectively. There was also an increase in antioxidative enzyme genes, manganese superoxide dismutase (SOD2) and glutathione peroxidase. These changes were considered a cell response mechanism against diabetic oxidative stress and to compensate the loss of UCP proteins under diabetic conditions. Pioglitazone increased UCP-3 but not UCP-2 in the non-diabetic conditions showing it enhances UCP-3 expression in DRG neurons. Pioglitazone further upregulated UCP-3 in diabetic conditions.
Conclusion: Pioglitazone ameliorated diabetic neuropathy. The mechanism was considered at least in part through UCP-3 upregulation and increased antioxidant defense.
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Are patient characteristics predictive of a response to pregabalin? Analysis from 5 randomized, placebo-controlled trials in painful diabetic peripheral neuropathy

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Background and Aims: Pregabalin has consistently demonstrated rapid and robust efficacy for the treatment of

peripheral neuropathic pain syndromes and associated sleep interference. We investigated whether selected patient characteristics might be predictive of responder rates greater than that for the total population of patients from 5 pregabalin RCTs (of 5–12 weeks) in painful diabetic peripheral neuropathy (DPN).

Materials and Methods: In these 5 trials, pregabalin-treated patients received dosages of 150 (n=175), 300 (n=252), or 600 (n=347) mg/d; 421 patients received placebo. Patients were considered responders if they had ≥30% reduction from baseline to endpoint in mean pain score (11-point scale). Using ARF (data-mining trees) software, we searched for subregions of treatment group, age, gender, baseline creatinine clearance (CL_{cr}), pain score or sleep interference score, and Patient Global Impression of Change (PGIC) that had high responder rates.

Results: The overall responder rate for the entire population (including placebo patients) was 33%. Sixteen subsets of patients demonstrated significantly greater responder rates than the overall responder rate. Treatment with 300 or 600 mg/d (n=568) as opposed to treatment with 150 mg/d or placebo was associated with a responder rate of 42%. Across treatment groups, a PGIC rating of "much improved" or "very much improved" was consistently associated with greater percentages of patients meeting the definition of responder. Baseline characteristics eg, gender, age, CL_{cr}, and pain and sleep scores were not consistently predictive of greater likelihood of response to pregabalin.

Conclusion: Pregabalin particularly at dosages of 300 or 600 mg/d provided clinically meaningful pain relief for patients with painful DPN. Response to pregabalin appeared to be largely independent of baseline characteristics.

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Pregabalin safely and efficaciously treats painful diabetic peripheral neuropathy without affecting nerve conduction: a double blind, placebo controlled trial

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Background and Aims: Pregabalin is approved in the EU for the treatment of peripheral neuropathic pain. This 13 week trial evaluated nerve conduction and the efficacy and safety of pregabalin 600 mg/d (BID) for relieving neuropathic pain associated with diabetic peripheral neuropathy (DPN).

Materials and Methods: Amplitude and conduction velocity were assessed at baseline (BL), endpoint (EP), and 2 week follow up for 4 nerves: median motor, median sensory, ulnar sensory, and peroneal motor. The study's primary efficacy measure was EP mean pain score (MPS) on an 11 point numeric rating scale (0="no pain" to 10="worst possible pain") derived from patients' daily pain diaries. Secondary efficacy measures included weekly MPS and proportion of responders (patients with ≥50% reduction in MPS from BL to EP). Eighty two patients were randomized to pregabalin and 85 to placebo. Most were male (62%) and white (74%); mean age was 58 years (range, 31–86); 92% had type 2 diabetes; mean durations of diabetes and painful DPN were 10 and 5 years, respectively. After a 1-week titration, pregabalin patients received 300 mg (BID) for 12 weeks.

Results: For all 4 nerves, 95% CIs for median differences in amplitude and velocity from BL to EP and to follow-up included 0. At EP, pregabalin patients' MPS was significantly lower than placebo patients' (mean difference, −1.28; $P<0.001$). Significant pain improvement among pregabalin patients was evident at Week 1 and was sustained at every weekly timepoint. Forty-nine percent of pregabalin-treated patients vs 23% of placebo patients were responders ($P<0.001$).

Conclusion: Pregabalin had no statistically significant or clinically meaningful effect on nerve conduction. BID dosing at the upper end of pregabalin's effective dosing range (150–600 mg/d) showed robust efficacy and was well tolerated.

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Amelioration of diabetic peripheral neuropathy by implantation of haematopoietic mononuclear cells in streptozotocin-induced diabetic rats

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Background and Aims: This study was performed in order to evaluate the angiogenic effect of implantation of either peripheral blood mononuclear cells (PBMNCs) or bone marrow mononuclear cells (BMMNCs) on diabetic peripheral neuropathy.

Materials and Methods: Streptozotocin (50 mg/kg) was injected intravenously into six-week-old male Lewis rats. Four weeks after the induction of diabetes, 6×10^7 of PBMNCs or 1×10^8 of BMMNCs were implanted into the left hindlimb muscle. Motor nerve conduction velocity

(MNCV) was monitored before and after implantation. At the end of the experiment, bilateral nerve blood flow (NBF) was measured by laser Doppler and the number of vessels in the sciatic nerves quantified by Factor VIII staining of the sections.

Results: Diabetes resulted in an approximately 20% reduction ($p<0.01$) in sciatic MNCV. Four weeks after implantation, MNCV was improved by 54% with PBMNCs, and by 67% with BMMNCs (both $p<0.01$). Moreover, the effects of implantation were almost abolished by administration of VEGF-neutralizing antibody. Sciatic NBF was reduced by approximately 50% by diabetes ($p<0.05$). This reduction in perfusion was improved by 74% by implantation of PBMNCs and by 62% by implantation of BMMNCs ($P<0.05$ and $p<0.01$, respectively). These effects were observed only in the implanted limb. Immunohistochemical staining of sciatic nerve sections for Factor VIII showed no significant increase in the number of vessels in the sciatic nerve following implantation of either PBMNCs or BMMNCs. These data suggest that implantation of hematopoietic mononuclear cell fractions is associated with an improvement in MNCV as a result of arteriogenic effects in the sciatic nerve and that VEGF may contribute to this effect. This improvement occurred in the absence of angiogenesis.

Conclusion: Implantation of these cell fractions may therefore be a potential new therapeutic method for treating diabetic peripheral neuropathy.

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Single treatment, phase 1 trial of a zinc finger DNA-binding protein (ZFP) activator (SB-509) of vascular endothelial growth factor (VEGF) in subjects with diabetic neuropathy

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Background and Aims:**:** This is a single treatment, dose escalation trial for evaluation of the clinical and laboratory safety of SB-509, a formulation of a plasmid DNA expressing an engineered zinc finger DNA-binding protein transcription factor (ZFP TF) activator of VEGF, in subjects with diabetic neuropathy (DN).This ZFP TF for the VEGF gene has been developed to induce expression of all isoforms of VEGF. VEGF is a growth factor for both neurons and glial cells. Pre-clinical studies in the Rat Streptozotocin diabetic neuropathy model with SB-509 has shown improved nerve conduction.

Materials and Methods: Patients with mild to moderate diabetic neuropathy were enrolled. The clinical trial design was a dose escalation Phase 1 trial with 4 dose levels, 1 mg, 5 mg, 15 mg and 30 mg. SB-509 was given intramuscularly in one leg and placebo intramuscular injections were given in the other leg. In addition, the trial was amended to include patients treated with either placebo or SB-509 in both legs at dose levels of 30 and 60 mg. Patients were followed for 6 months for clinical and laboratory safety, Total Neuropathy Score (combining neurologic sensory, motor, reflex exam, QST and NCV measurements), visual analog scales for pain (VAS), Quantitative Sensory testing by Vibratron II (QST).

Results:**:** Eighteen patients have been enrolled and evaluablel for safety and twelve patients are evaluable for clinical endpoints. Adverse events were mild and primarily related to injection site reactions. No dose limiting toxicities (DLT) or serious adverse events (SAE's) were seen. Improvements in symptoms of pain (2 points VAS) were seen in 6/12 patients and numbness in 6/12 patients. TNS composite scores improvements were seen in 6/12 patients and a decrease in vibration threshold sensitivity by QST (2 point) were seen in 7/12 patients.

Conclusion: Single dose treatment of SB-509 was well tolerated to doses of 60 mg in both legs with no DLT or SAE's. Encouraging anecdotal improvements in symptoms of pain and numbness, measured TNS scores and QST measurement were noted and suggest further evaluation in placebo controlled trials. Improvements in pain symptoms suggest a novel endpoint for ZFP TF VEGF activator treatment in Diabetic Neuropathy. A Phase 2 controlled trial of SB-509 versus placebo is planned for 2006.

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Duloxetine in the management of diabetic peripheral neuropathic pain: Response profile

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Introduction: Analyses examine the response rate at endpoint, as well as time course of response, in patients receiving duloxetine for management of diabetic peripheral neuropathic pain (DPNP).

Methods: Data were pooled from 3 double-blind, randomized, placebo-controlled 12-week trials in patients with DPNP ≥6 months duration, and without depression. Study 1 (N=457) compared duloxetine 20 mg once daily (QD), 60 mg QD, 60 mg twice daily (BID), and placebo; Studies 2

(N=334) and 3 (N=348) compared duloxetine 60 mg QD and 60 mg BID with placebo. Ethics committees approved the study protocol in accordance with Declaration of Helsinki principles. Patients provided written informed consent prior to study participation. Treatment response was defined a priori as 30% reduction in the primary efficacy measure, 24-hour average pain severity. Analysis was replicated using alternative criteria (50% reduction or 2-point reduction).
Results: Endpoint response rates were significantly higher among patients receiving duloxetine (60 mg QD or 60 mg BID) than those receiving placebo, regardless of chosen response criterion. The proportion of patients responding (30% reduction in 24-hour average pain severity) to duloxetine was statistically greater than to placebo Week 1 and all subsequent visits. Similar results were obtained for the visitwise sustained response rate. Within the group with a sustained response at Week 12, the proportion of patients first exhibiting a response at Weeks 1 or 2 was higher in the duloxetine groups (60 mg QD, 65.0%; 60 mg BID, 62.0%) when compared with the placebo group (40.2%).
Conclusion: Patients with DPNP receiving duloxetine 60 mg QD or 60 mg BID had significantly higher treatment response rates compared with patients receiving placebo, regardless of response criterion. Response to duloxetine treatment tended to occur early, with approximately 30% of patients responding at Week 1. Among duloxetine-treated patients with a sustained treatment response at Week 12, over 60% had maintained responder status for 10 to 11 weeks.
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Duloxetine's long-term effects on glycaemic control in patients with diabetic peripheral neuropathic pain

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Duloxetine has been shown to be safe and effective in the management of diabetic peripheral neuropathic pain (DPNP). We report the impact of long-term treatment with duloxetine on fasting blood glucose (FBG), glycosylated hemoglobin (HbA1C), lipids and weight.
In three long-term studies, 867 patients were randomized at 2:1 ratio to duloxetine 60 mg BID (N=580) or routine care (N=287) for 52 weeks after completing 12-week placebo-controlled, acute phase studies. Pooled data from the study extensions were analyzed.
After long-term treatment with duloxetine of up to 52 weeks, there was an increase in HbA1C in both the duloxetine and routine care groups, but the mean increase was greater in the duloxetine-treated group (0.5% vs 0.2%, respectively, $p<0.001$). There was also a modest increase in FBG and a small increase in total cholesterol in duloxetine-treated patients while the routine care group showed a slight decrease (mean change FBG: 0.67 vs −0.64 mmol/L, $p<0.001$; cholesterol: 0.06 vs −0.16 mmol/L, p=.005, respectively). A small decease in HDL cholesterol was seen in both groups, although the decline was significantly smaller with duloxetine than with routine care (mean change: −0.014 vs −0.078 mmol/L, p=.002 respectively). In these trials, after 52 weeks of therapy, the weight increase was smaller in the duloxetine-treated patients, compared to routine care group, but the difference was not statistically significant. The frequency of diabetes-related adverse events was similar between groups.
Long-term duloxetine treatment appears to be associated with a slight worsening of metabolic control in patients with DPNP. Patients managed with duloxetine for DPNP should continue to have their glycemic control closely monitored.
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Treatment of painful diabetic neuropathy with Sativex (a cannabis based medicinal product) - results of a randomised placebo controlled trial

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Background: Painful diabetic neuropathy (PDN) affects 10% of subjects with diabetes and about a third with diabetic neuropathy. Adjuvant drug therapies to alleviate painful symptoms are often ineffective and associated with side-effects. Cannabis in its various guises has been consumed for its pain relieving properties for many centuries. Sativex is an oromucosal spray preparation containing the therapeutic components of cannabis (2.7 mg/ml tetrahydrocannabinol, 2.5 mg/ml cannabidiol per actuation) and has provided symtomatic relief in other conditions of chronic pain.
Aims: To assess the efficacy of a 12 week multiple daily dosing of Sativex added to standard therapies in improving primary (pain) and secondary (quality of life) end points in adult subjects with PDN.
Methods: In this randomised, double-blind, placebo controlled trial, 30 subjects with intractable PDN unresponsive to currently available treatments were recruited. They were randomised to receive either Sativex or placebo as an oromucosal spray. The primary outcome measure was mean

daily pain intensity in subjects receiving a maximum tolerated dose. Pain symptoms was assessed using a multi-dimensional daily pain diary. The secondary outcome measure was quality of life as assessed by 1) the McGill Pain and Quality of Life Questionnaires, 2) the SF-36 Health survey and 3) the EuroQOL Health Utilities Index.
Results: There was a substantial and significant improvement in pain scores in both groups over the course of the study. There was, however, no significant difference between the two treatment arms for the primary outcome measures. The change in pain from baseline (mean+SD), based on a 0–10 cm visual analogue scale for deep pain, muscular pain and superficial pain, at the maximum tolerated dose of the study drug is shown in Table 1. There was also no significant differences between Sativex and placebo in the secondary outcome measures for quality of life.

Table 1. Change in pain scores during study

	Sativex (mean(SD))	**Placebo** (mean(SD))	**P-value**
Total Pain	−1.66 (2.4)	−1.91 (3.0)	0.50
Superficial Pain	−1.56 (1.9)	−1.52 (3.2)	0.14
Deep Pain	−2.00 (2.9)	−2.21 (2.9)	0.97
Muscular Pain	−1.50 (3.0)	−2.00 (3.3)	0.49

Conclusion: This first ever randomised placebo controlled trial assessing the efficacy of a cannabis based medicinal product (Sativex) has shown it to be no more efficacious than placebo in the treatment of painful diabetic neuropathy. The larger than expected placebo response, which has also been demonstrated in other recent studies, may provide us with a important clue as to the nature of pain in diabetic neuropathy. It may also have important implications for the design of future trials in the treatment of painful diabetic neuropathy.
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Autonomic neuropathy - cardiovascular

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Prospective evaluation of myocardial sympathetic innervation defects and autonomic function testing in relation to glycaemic control over 12 years in type 1 diabetic patients

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Background and Aims: Reduced or absent myocardial [^{123}I]metaiodobenzylguanidine (MIBG) uptake is a frequent feature of diabetic cardiovascular autonomic neuropathy (CAN), but the long-term progression of myocardial adrenergic innervation defects in relation to changes in autonomic function tests (AFTs) and glycaemic control is not known. We aimed to determine the role of glycaemic control in the long-term progression of these defects in a 12-year prospective study.
Materials and Methods: We examined left ventricular myocardial sympathetic innervation in 12 long-term Type 1 diabetic patients (age [mean±SD]: 41.4±10.4 years, diabetes duration: 22.6±8.8 years) using MIBG scintigraphy. Global and regional relative MIBG uptake were assessed and MIBG defects at 2 h p.i. were scored by 3 scorers at baseline and after 4 and 12 years, respectively. AFTs were performed using a standardized test battery including time domain and frequency domain measures of heart rate variability. Two patients died during follow-up. Among the remaining patients 6 were well controlled (mean HbA_{1c} over 12 years: 7.1±0.4%; Group 1), whereas 4 patients were poorly controlled (mean HbA_{1c} over 12 years: 9.0±1.1%; Group 2).
Results: In the entire cohort both myocardial MIBG uptake defects of all 9 regions summed to a score did not change significantly from baseline to 12 years (13.1±2.8 vs 12.4±4.8) and, likewise, the relative MIBG uptake remained unchanged (78.8±8.0% vs 79.2±6.0%). No significant differences were noted between the well and poorly controlled patients. In contrast, regional MIBG uptake score in the posterior wall increased by 0.28±0.48 from baseline to 12 years in Group 1 and decreased by −0.23±0.15 in Group 2 ($p<0.05$). Among the AFTs, measures of parasympathetic activity such as the mean circular resultant during deep breathing declined significantly from 0.021±0.014 to 0.015±0.011 ($p<0.05$) and the maximum/minimum 30:15 ratio tended to progress from 1.16±0.19 to 1.05±0.03 ($p=0.05$) in the entire cohort, without significant differences between the two groups of glycaemic control.
Conclusion: Global myocardial adrenergic innervation defects do not progress over 12 years in Type 1 diabetic patients, despite continuing progression of vagal dysfunction. However, regional progression of sympathetic dysinnervation in the posterior wall may be slowed by long-term near-normoglycaemia.

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Cardiac autonomic dysfunction and inflammation in type 1 diabetic patients. Effect of beta-blockade

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Background: Recent studies suggest a relation between abnormal nervous autonomic function and inflammation. Impaired cardiac autonomic activity and inflammation have both been found to be associated with a worse outcome in diabetic patients. However, whether the two abnormalities are related in these patients is unknown.
Methods and Results: We studied 40 patients with type 1 diabetes mellitus (age 50.6±10 years, 25 men). Serum levels of high-sensitivity C-reactive protein (CRP) and 24-hour Holter monitoring to assess frequency-domain heart rate variability (HRV), as a measure of cardiac autonomic function, were obtained in all patients. Seventeen patients with depressed HRV were subsequently randomized to receive atenolol (50 mg daily) or no beta-blockade. HRV and CRP were reassessed after 4 weeks from randomization. A significant inverse correlation was found between CRP levels and HRV parameters, with the highest r coefficient shown with low frequency (LF) amplitude ($r=-0.47$.; $P=0.008$). On multivariate logistic regression analysis only low LF amplitude ($P=0.037$) and high body mass index ($P=0.05$) were independent predictors of high (>3 mg/L) CRP serum levels. HRV parameters improved significantly in patients treated with atenolol, but not in the no-atenolol group. CRP also tended to decrease in the former group ($P=0.10$), but not in the latter.
Conclusion: In type 1 diabetic patients, serum CRP levels are significantly associated with depressed HRV; the favourable effects of beta-blockade on both HRV parameters and CRP serum levels suggest that autonomic nervous system can be an important modulator of inflammation.

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Validation of a new diabetic autonomic neuropathy bedside test (plaster) versus the cardiovascular reflex tests

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Background and Aims: Autonomic neuropathy is a serious and fatal complication of Diabetes Mellitus (DM). Perspiration is a feature of Diabetic Autonomic Neuropathy **(DAN)** and the Indicator Plaster **(IP)** could provide an easy and accurate way to diagnose DAN especially for general practitioners. The aim of the present study was to evaluate indicator's specificity, sensitivity and accuracy in detecting DAN.
Materials and Methods: We studied 174 patients (79 with DM type 1, 88 women), with mean age 49.8+16.1 years and mean duration of DM. 17.3+7.7 (range 4–45 years). We performed the following four Cardiovascular Reflex Tests (CRT): R-R variation during deep breathing [assessed by Expiration/Inspiration ratio (E/I), Mean Circular Resultant (MCR, vector analysis) and Standard Deviation (SD)], Valsalva maneuver, 30:15 ratio and postural hypotension in all patients. The presence of DAN was established if 2 or more 4 CRT were abnormal. One test abnormal considered as early DAN According to the change of color of the sticker IP or not patients divided in two groups: Group A (n=82, completed change in color from pink to blue, normal perspiration), and Group B (n=92, uncompleted or no change, abnormal perspiration).
Results: According to the number of abnormal tests, established DAN detected in 81 patients. E/I index was abnormal in 118 patients and postural hypotension in 73. The sensitivity, specificity and accuracy of IP versus CRT of the above measured parameters appear in the following table.

Measured Parameter	Sensitivity	Specificity	Accuracy
Established DAN	89%	78%	84%
E/I index	73%	89%	85%
Postural Hypot	95%	77%	85%

Conclusion: Indicator has a high sensitivity, specificity and accuracy in detecting established DAN versus CRT. Neuropathy of parasympathetic origin was observed with IP with low sensitivity, but high specificity. Neuropathy of sympathetic origin was detected with IP with high sensitivity and specificity. Accuracy of the IP test was high in all measurements.

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How two different diagnostic criteria of orthostatic hypotension relate to the presence of cardiac autonomic neuropathy or nondipping in type 1 and type 2 diabetes

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Background and Aims: There is not yet a widely accepted approach to the diagnosis of orthostatic hypotension (OH) in diabetes. Thus, we evaluated the diagnostic accuracy for autonomic neuropathy (AN) and for blood pressure (BP) nondipping of OH defined according to two distinct criteria, i.e. a fall in systolic BP >30 mmHg (30-OH) or >20 mmHg (20-OH).
Materials and Methods: We studied 84 type 1 (age 36±11, duration 17±10 years, serum creatinine 0.76±0.18 mg/dl,

casual BP 119/73±14/9 mmHg), and 80 type 2 diabetic patients (age 51±11, duration 11±8 years, serum creatinine 0.74±0.19 mg/dl, casual BP 127/77±15/9 mmHg), all with normal renal function and without current antihypertensive treatment. We assessed the presence of orthostatic symptoms and performed 24 h BP monitoring and 4 cardiovascular tests (deep breathing, lying to standing, Valsalva manoeuvre, and postural hypotension test measuring BP supine and following standing at 1 and 2 min).

Results: Among type 1 patients, 30-OH and 20-OH were present in 9.5% and 20.2% of patients, respectively. Orthostatic symptoms were present in 29.6% without a complete overlapping with OH (for 30-OH: Chi^2=8.8, p=0.007; for 20-OH: Chi^2=10.3, p=0.004). Both 30-OH and 20-OH were associated with AN, defined as the presence of at least 1 abnormal heart rate test (Chi^2=6.6, p=0.009, and Chi^2=5.5, p=0.03, respectively), and with nondipping, defined as a nocturnal fall in systolic or diastolic BP<0% (Chi^2=13.4, p=0.003, and Chi^2=5.3, p=0.03, respectively). Using ROC analysis, systolic BP fall on standing showed a rather low diagnostic accuracy in discriminating between patients with and without AN (AUC 0.62±0.06, 95% C.I. 0.50–0.73), and a fair accuracy in discriminating between patients with and without nondipping (AUC 0.75±0.08, 95% C.I. 0.58–0.88). Both 30-OH and 20-OH showed low sensitivity and high specificity for AN [for 30-OH: sensitivity 17%, specificity 100%, positive predictive value (PPV) 100% (95% C.I. 100–100), negative predictive value (NPV) 47% (95% C.I. 36–58); for 20-OH: sensitivity 29%, specificity 92%, PPV 82% (95% C.I. 64–100), NPV 49% (95% C.I. 37–61)]. 30-OH showed a slightly better PPV for nondipping [sensitivity 36%, specificity 96%, PPV 62% (95% C.I. 29–96), NPV 88% (95% C.I. 81–95)] than 20-OH [sensitivity 43%, specificity 84%, PPV 35% (95% C.I. 13–58), NPV 88% (95% C.I. 80–96)]. Among type 2 patients 30-OH and 20-OH were present in 12.5% and 18.7% of patients, respectively. Orthostatic symptoms were present in 24% of patients with weak or no association with 30-OH (Chi^2=4.2, p=0.05) or 20-OH (Chi^2=2.6, p=0.18). Also in type 2 patients 30-OH and 20-OH were associated with AN (Chi^2=8.8, p=0.005, and Chi^2=12.1, p=0.001, respectively), or nondipping (Chi^2=17.9, p=0.0003, and Chi^2=12.8, p=0.001, respectively), with values of AUC, sensitivity, specificity, PPV, and NPV similar to those observed in type 1 patients.

Conclusion: There is no strict overlapping between orthostatic symptoms and OH, in particular in type 2 patients. Both 30 mmHg and 20 mmHg criteria display low sensitivity and high specificity in diagnosing cardiac AN. The 30 mmHg criterion has a better predictive value for nondipping as opposed to 20 mmHg. Since the presence of postural hypotension is burdened by a further increased adverse prognostic impact with regard to AN, the more restrictive 30 mmHg criterion could be likely preferred in diabetic patients.

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Disturbances of spectral heart rate variability during tilting in patients with diabetes mellitus type 1

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Background and Aims: The autonomic regulation is impaired in patients with diabetes mellitus. Ortostatic hypotension (OH) in patients with diabetes is one of the sign of autonomic dysfunction. Analysis of heart rate variability (HRV) is regognized method to evaluate cardiac autonomic neuropathy. The aim of this study was to assess blood pressure and spectral HRV. parameters and evaluate during 5 minutes tilting in patients with type 1 diabetes mellitus (DM1).

Materials and Methods: We studied 48 normotensive patients with DM1 aged 18–28 years, with 9–23 years of DM1. Control group consisted of 32 healthy persons, matched to the DM1 group. We performed 24 h Holter ECG monitoring (SUPRIMA 12, DMS, USA) and ABPM (Spacelab 90207, Datex-Ohmeda, USA). We initialized manually measurements every minute during 5 minutes standing up, preceded by 15 minutes of rest in supine position. Furthermore we analyzed spectral HRV parameters: total power (TP), very low frequency (VLF), low frequency (LF) and high frequency (HF) - in 5 minutes periods in supine and standing position.

Results: According to the adopted diagnostic criteria of orthostatic hypotension (OH) we noted systolic blood pressure (sBP) fall in 19% patients with DM1 and 3% persons in control group (p=0.001) and diastolic blood pressure (dPB) fall in 23% patients with DM and 0% in control group (p=0.001). In rest conditions (lying position) patients with DM and OH, in comparison to healthy persons and patients with diabetes without co-existent OH, showed significant lower TP and VLF spectrum (p=0.001).

After tilting we observed lower values of TP, VLF, LF and HF in group of patients with DM and HO versus control (p=0.001). Moreover in standing position we registered lowering of LF component, measured in normalized unit (nu) in patients with DM (with and without OH) in contrast to healthy people, in which LF spectrum increased.

Conclusions: OH is a frequent pathology in young patients with long-lasting DM1. Patients with DM and OH have more advanced of autonomic dysfunction then DM patients without OH, manifested of lower spectral HRV components during tilting. DM patients show pathological fall LF (nu) component during tilting.

1110

Correlation between autonomic dysfunction and arterial stiffness in patients with long-standing diabetes mellitus

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Background and Aims: The *augmentation index* (AIx80), a ratio calculated from the first pulse wave generated in the aorta after left ventricular contraction and the second pulse wave reflected from the aortic bifurcation, provides information regarding both the aortic elasticity and the peripheral arteriolar resistance. A higher than normal value of AIx80 is mainly indicative of an elevated peripheral resistance. On the other hand, an accelerated *pulse wave velocity* (PWVS35) is primarily characteristic of aortic stiffness. It is well known that the level of *arterial stiffness* is elevated in diabetic patients. The aim of the present study was to evaluate the correlations between the arterial stiffness characterized by AIx80 and PWVS35 and the results of standard cadiovascular reflex tests (CRTs) in patients with long-standing diabetes mellitus (DM).
Patients and Methods: 22 patients with long-standing DM (16 women and 6 men, age: 47.5±3.3 years, duration of DM: 15.3±2.0 years, BMI: 26.2±1.0 kg/m^2, HbA1c: 8.1± 0.4%, systolic BP: 147.6±3.5 mm Hg, diastolic BP: 84.1± 2.2 mm Hg; mean±SEM) were enrolled into the study. The autonomic function was assessed by means of five standard CRTs, the results of which were utilized to create a score with which to express the severity of autonomic neuropathy (AN score). AIx80 and PWVS35 were measured non-invasively with a TensioClinic TL1 arteriograph and PC-based software. Pearson correlation coefficients (r) were evaluated between AIx80, PWVS35 and the CRT parameters.
Results: AIx80 revealed a close correlation with the AN score (r=0.72, p=0.00015), the 30/15 ratio (r=−0.60, p=0.003), the heart rate response to deep breathing (r=−0.64, p=0.0012), the Valsalva ratio (r=−0.56, p=0.006) and the systolic blood pressure response to standing (r=0.56, p=0.007). PWVS35 correlated with the AN score (r=0.44, p=0.038) and the heart rate response to deep breathing (r=−0.53, p=0.011). There was no significant relationship between AIx80, PWVS35 and HbA1c.
Conclusion: Our results suggest the existence of a close relationship between the enhanced arterial stiffness characterized by the augmentation index and by the pulse wave velocity and the degree of autonomic dysfunction in patients with long-standing diabetes mellitus.
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Continous variations of glucose levels in type 2 diabetic patients are accompanied by corresponding changes in heart rate variability

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Background: Heart rate variability (HRV), a marker of autonomic dysfunction, is known to be impaired in type 2 diabetic patients and tight blood glucose control has been reported to improve HRV parameters in these patients. Recent data deriving from continuous glucose measurement in animal models of diabetes mellitus have suggested tha HRV may phasically influenced by blood glucose fluctuations. Whether this potentiallity relevant dynamic is present in humans is unknown.
Methods: Twelve patients with type 2 diabetes mellitus (mean age 65±4 years, 2 women) underwent 48-hour Holter monitoring and continuous interstitial glucose measurement by amperometric method with a needle electrode (Medtronic/MiniMed) placed subcutaneously in the abdomen and acquiring data every 5 minutes. The higher and lower glucose levels for each 3-hour segment of the day and their time of detection were identified and RR interval and HRV parameters were calculated on a 5-minute interval centred on these times. HRV was assessed both in the time-domain (average value of the 5-minute standard deviations [SDNN-I], root-mean squared successive difference [r-MSSD], the percentage of RR intervals differing by ≥50 msec from the previous interval [pNN50] and in the frequency-domain (low-frequency amplitude [LF], high-frequency amplitude [HF], and LF/HF ratio).
Results: Overall, 303 glucose levels were available for analysis. A significant correlation was found between glucose levels and several HRV parameters as r-MSSD (p<0.003), pNN50 (p<0.001), LF (p<0.03), and HF (p<0.01).

Conclusions: Our data suggest that, in type 2 diabetic patients, daily fluctuations in glucose levels are associated with parallel changes in HRV parameters. Specifically, HRV changes towards decrease in vagal activity, with adrenergic predominance, are associated with lower glucose levels. The pathophysiologic and clinical implications of these findings deserve further investigation.

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Acute effects of sildenafil on flow mediated dilatation, haemodynamic parameters and cardiovascular autonomic function in type 2 diabetic men

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Background and Aims: Sildenafil, a selective inhibitor of the cGMP-specific phosphodiesterase type 5 (PDE5) isoenzyme, is widely used in patients with type 2 Diabetes mellitus (T2DM) in the treatment of erectile dysfunction (ED). Evidence has emerged that sildenafil may exert effects on various vascular and autonomic nervous systems other than the corpus cavernosum. Because both endothelial function and the autonomic nervous system integrity are frequently disturbed in Type 2 diabetic patients, we aimed to assess the effects of sildenafil on flow mediated dilatation (FMD) and cardiovascular autonomic nerve function in men with T2DM.
Materials and Methods: A double-blind, placebo-controlled cross-over trial has been conducted in 35 men with T2DM without known cardiovascular disease (CVD). Patients' characteristics were (mean±SEM): age: 55.1± 1.17 yrs; diabetes duration: 6.4±0.8 yrs, BMI: 30.8± 0.8 kg/m^2; HbA1c: 7.9±0.3%. Diabetes complications were present as follows: ED: 91%; peripheral neuropathy: 34%, cardiac autonomic neuropathy: 17%, microalbuminuria: 17%, and retinopathy: 14%. We assessed in these patients the acute effect of 100 mg Sildenafil on FMD of the brachial artery as a measure of endothelial dysfunction, blood pressure (BP) at rest and its response to standing up, heart rate (HR), heart rate variability (HRV) using time domain and frequency domain indices, and spontaneous baroreflex sensitivity (BRS).
Results: Sixty minutes after a single oral administration of sildenafil, a decrease in supine systolic (−5.41±1.87 mmHg,) and diastolic (−4.46±1.13 mmHg) BP, as well as systolic (−7.41±2.35 mmHg) and diastolic (−5.65± 1.45 mmHg) BP on standing were observed, accompanied by an increase in heart rate (+1.98±0.69 beats/min) (all $p<0.01$ vs. placebo). No acute effects of sildenafil were noted on parasympathetic and sympathetic nervous activity as assessed by HRV and BRS. FMD variations from baseline to 60 min after medication intake were not statistically significant after any intake: 4.13±0.28% to 3.67±029%‡ (sildenafil) and 4.08±0.31% to 4.42±0.31%‡ (placebo) (‡ p=NS vs. corresponding baseline).There was no significant difference between sildenafil and placebo regarding the changes in FMD.
Conclusion: In men with T2DM without clinical evidence of CVD, a single oral administration of 100 mg sildenafil leads to a decrease in blood pressure and an increase in heart rate, but not to an adverse, potentially arrhythmogenic effect on cardiovascular autonomic function with increased sympathetic and decreased vagal activity. However, no acute effect of sildenafil on endothelial dysfunction could be demonstrated.
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Erectile dysfunction and cardiovascular risk in male patients with type 2 diabetes in primary care

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Background and Aims: The prevalence of questionnaire-diagnosed erectile dysfunction (ED) in primary care is 30–50%.The prevalence in diabetic men varies from 20–70% and increases with age. ED questionnaires are too time consuming in daily practice. However, the ED prevalence rate in primary care diagnosed by a single question is unknown.
Patients with Diabetes Mellitus, cardiovascular disease (CVD) or markers of poor cardiovascular prognosis have more often ED. ED is a strong predictor of silent coronary artery disease in diabetic patients.
We aim to study the single question prevalence of ED in men with type 2 diabetes (DM2) in general practice (GP) and the association with cardiovascular risk-factors.
Materials and Methods: In a cross-sectional study in 54 practices, all male DM2 patients treated by their GP were asked by a female practice nurse if they had erection problems (yes/no). Gender, ethnicity, smoking, duration of diabetes, HbA1c, systolic blood pressure, cholesterol and HDL-cholesterol were used to calculate the 10 year UKPDS coronary heart disease risk score. Medication, Simm's-classification for the diabetic foot as a sign of microvascular complications (Simm's 2 and 3 vs 0 and 1),

cardiovascular complaints and diabetic complications (yes/no) were co-determinants. Prevalence of ED in DM2 men and its univariate associations with risk factors were analysed by frequency, cross-tabulation and Mann-Whitney's test. In a multivariate logistic regression analysis we adjusted for the co-determinants.

Results: 1675 DM2 men consulted the practice nurse, ED was registered in 1476 patients. The prevalence of ED was 41.3%, increasing with age. These men had more often cardiovascular complaints (odds ratio, OR 1.6; 95% confidence interval, 95% CI 1.3–2.0), diabetic complications (OR 2.9; 95%CI 2.3–3.7) and foot complications (OR 1.6; 95% CI 1.1–2.2). The mean 10 year UKPDS risk score was 29.5% and 24.0% for men with and without ED, respectively ($p<0.001$). The mean HbA1c in both groups was 7.1% and 7.0% ($p=0.01$), systolic blood pressure 150 mmHg and 146 mmHg ($p=0.003$), and for duration of diabetes 6.3 years and 5.4 years ($p=0.001$), respectively. For smoking, total cholesterol, HDL cholesterol, BMI and diastolic blood pressure the difference was not statistically significant.

Conclusion: In general practice 41.3% of DM2 men have ED as diagnosed by a single question, this is comparable to questionnaire based prevalence. These men have a 5.5% higher 10 year UKPDS cardiovascular risk estimate and more cardiovascular complaints, diabetic complications and microvascular foot complications, irrespective of cholesterol, HDL-cholesterol, BMI and diastolic blood pressure. Time consuming questionnaires may not be necessary for identifying DM2 men with ED. Asking for ED identifies patients at high cardiovascular risk.

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Autonomic neuropathy - gastrointestinal

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Determination of gastric emptying and neuropathy status in patients with different durations of type 1 diabetes

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The roles of the disease duration and age in the pathogenesis of impaired gastric emptying in type-1 diabetes (DM) are still not clearly explored. The **background and aims** of this study were to determine the postprandial gastric emptying, the autonomic and the sensory neuropathy in groups of diabetic patients (pts) with different durations and age.

Patients, methods: 17 patients had a shorter, 23 pts had a longer history of type-1 DM (age: 30.7±2 *vs* 44.6±2.6 yrs, $p<0.001$; duration: 8±1.4 *vs* 25.3±2.3 yrs, $p<0.0001$; mean± SE). The emptying of the stomach was evaluated by a scintigraphic gastric emptying procedure. Autonomic neuropathy (AN) was assessed by means of the five cardiovascular reflex tests. The sensory nerve function was studied with a Neurometer (Neurotron Inc. Baltimore, Md) using transcutaneous electrical stimulation.

Results: There was a tendency to a longer half-time of gastric emptying (HTE) in type-1 DM pts with longer duration, but the difference between the groups did not reach the level of significance (HTE: 93.5±10.1 *vs* 67.8± 6.7 min., $p>0.05$; long-standing *vs* short-standing type-1 DM). The overall severity of AN and the results of three reflex tests differed between the groups (AN score: 4.3±0.5 *vs* 2.5±0.4, $p<0.05$; heart rate response to breathing: 12.7± 1.5 *vs* 19.4±2.2 beats/min., $p<0.05$; Valsalva ratio: 1.3±0.05 *vs* 1.75±0.1, $p<0,0001$; orthostatic hypotension: 23±3.4 *vs* 11±3.7 mm Hg, $p<0.05$). A positive correlation was observed between HTE and the AN score in long-standing DM ($r=0.57$, $p<0.01$). The correlations between heart rate response to breathing and HTE ($r=-0.57$, $p<0.01$) and between the Valsalva ratio and HTE ($r=-0.43$, $p<0.05$) in this group were negative, while a positive association was found between the orthostatic hypotension and HTE ($r=0.51$, $p<0.01$). The current perception thresholds (CPT) detected at 2 kHz frequency differed between the groups at the upper and lower limbs (CPT at the median nerve: 3.66± 0.35 *vs* 1.84±0.25 mA, $p<0,001$; CPT at the peroneal nerve: 5.39±0.71 *vs* 3.70±0.68 mA, $p<0.05$).

Conclusions: Impaired gastric emptying was found more frequently in pts with long-standing type-1 DM, but this difference was not significant between the groups. Both the parasympathetic and the sympathetic systems and the sensory function of the large myelinated fibres were more seriously altered in long-standing DM. The severe autonomic dysfunction exhibited a correlation with the slower gastric emptying. These data support the hypothesis, that the most progressive manifestations of diabetic neuropathy might be responsible for the development of gastroparesis.

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Intensive management with CSII can prevent hospital admissions of patients with gastroparesis

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Introduction: Gastroparesis is a difficult management problem that can have a significant impact on quality of life (QOL) and due to glycaemic instability it often leads to repeated hospital admissions.
A combination of hyperglycaemia, multiple motor and sensory abnormalities of the upper gut results in variable absorption of nutrients which leads to difficulties in controlling blood glucose levels with multiple dose injections (MDI). Individualised insulin delivery via continuous subcutaneous insulin infusion (CSII) may provide greater glycaemic stability in this group, which in turn may prevent acute complications and the necessity for in-patient management.
Aims: Prevent hospital admissions and improve QOL by stabilising glycaemic control in patients with gastroparesis by conversion from MDI onto CSII.
Methods: Seven female patients mean Age 32 years SD+/− 6.8 (range 24 –41) with a mean duration of type 1 diabetes of 20 years SD+/− 6.3 (range 12 –29) and symptomatic gastroparesis were converted from MDI onto CSII. All were on an optimised MDI regimen, which incorporated an average total daily insulin (TDI) of 44 units SD+/− 18.5 (range 24–78). Despite no educational deficits all had sub-optimal glycaemic control (mean HbA1c 10.5% SD+/− 1.3 (range 8.8 –12.2)), and erratic control which incorporated frequent episodes of hypoglycaemia, often requiring third party assistance, 5 describing total unawareness. Further autonomic complications included, 1 oesophageal, 3 bowel, 2 postural hypotension and 2 neurogenic bladder. Other established complications included, 5 retinopathy, 3 nephropathy (1 requiring dialysis), 2 hypertension, and 4 painful peripheral neuropathy. One hundred and seventy-four in-patient days in the preceding twelve months prior to CSII initiation were recorded. All reported low mood and 3 were treated with antidepressants.
Results: Mean duration of treatment with CSII was 15.7 months SD+/− 20.4 (range 2–60) and resulted in a reduction in the mean value of HbA1c by 1.9% and in TDI to a mean of 41.5 units SD+/− 9.2 (range 34.6–57.9). Hypoglycaemia was reported as infrequent and accountable and all episodes were associated with awareness. There was improvement of gastroparesis related symptoms and weight increased on average by 6.6 kg (mean BMI 23.3 SD+/− 3.5 (range 17–27.5). Well being and QOL was heightened with one patient discontinuing antidepressants. In-patient episodes related to glycaemic management or gastroparesis have not been required since conversion onto CSII.
Conclusion: CSII is an effective means of overcoming glycaemic instability caused by gastroparesis. Hospital admissions are reduced, symptom impact and QOL issues improve.

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Gastric neurostimulation ameliorates symptoms of diabetic gastroparesis

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Background: In its most unremitting form diabetic gastroparesis may present with abdominal pain and continous nausea and vomiting leading to hospitalisation for fluid substitution and blood glucose control. Fortunately the condition is often more limited, although frequently resistant to standard pharmacological and symptomatic treatment.
Method: Selected Type 1 diabetes patients underwent standard clinical examination excluding other causes of dyspepsia. All were before and after implantation of a gastric stimulator subjected to scintigraphic gastric emptying studies, visceral-biomechanical studies, 24 h-antroduodenal motility testing and qualitative symptomatic testing using a validated questionnaire.
Patients: Five patients, four males and one female, all Type 1 diabetes all long duration, all presenting with an array of late diabetic complications and attending the out-patient clinic at Department of Endocrinology M, Aarhus University Hospital. All had suffered symptoms of diabetic gastroparesis over several years resistant to standard treatment measures. All were frequently admitted to hospital for this condition.
Results: Three male patients have been relieved nearly totally of symptoms. One male patient is completely relieved of nausea and vomiting, but suffers abdominal cramps of unknown origin. One female demonstrates halving of admission frequency and is admitted for treatment of competing diabetic late complications. Some have not been admitted to hospital after implantation of the gastric neurostimulator, whereas they were frequently so before.
Conclusion: We conclude that the patients included in our study were significantly relieved of severe and disabling of diabetic gastroparesis.

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Acute exposure to palmitic acid damages autonomic enteric nerve in rat: preventive effects of protein kinase C inhibitor

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Background and Aims: Diabetes mellitus is often accompanied by elevated plasma free fatty acid levels. To determine if free fatty acid is an independent factor in the progression of diabetic gastroenteropathy, we evaluated functional changes in submucosal nerve plexus by acute exposure of rat colon to palmitic acid for representative substrate of free fatty acids. It is well known that protein kinase C (PKC) inhibitor prevents diabetic somatic nerve dysfunction. We evaluated the preventive effects of PKC inhibitor against damage of enteric nerve caused by palmitic acid.
Materials and Methods: Male Wister rats weighing about 200 g were used. Short-circuit current (Isc) in colonic mucosa was measured using an Ussing chamber. Stripped colonic mucosa (submucosal plexus intact) was mounted vertically between temperature (37 °C)-controlled Ussing chambers. Ringer's solution containing10 mM glucose was used as the standard bathing solution. All bathing solutions were gassed with a mixture of 95% O_2/5%CO_2, resulting in pH 7.4. All drugs were applied to the serosal bathing solution from the stock solution. The Isc response to electric-field-stimulation (EFS) was measured 0 and 30 minutes after exposure to 0.02 mM to 0.14 mM palmitic acid. To investigate the effect of PKC inhibitor, the colonic mucosa was pretreated with PKC inhibitor at a dose of 0.5 mM in the Ussing chamber before the application of 0.1 mM palmitic acid, and the Isc response to EFS was measured after 30 minutes.
Results: The Isc in response to EFS was dose-dependently decreased by exposure to palmitic acid, due to suppression of neuron-mediated Cl^- secretion (EC_{50} value and maximal decrease were 0.07 mM and 40%). Treatment with PKC inhibitor mitigated the suppression by exposure to 0.1 mM palmitic acid (4.9±0.6 vs. 7.1±1.1 μA/0.38 cm^2 at 30 minutes after treatment, $P<0.05$).
Conclusion: Acute exposure to palmitic acid clearly damaged autonomic enteric nerve, suggesting that palmitic acid is an independent factor in the progression of diabetic autonomic neuropathy, and indicates that PKC inhibitor may have a preventive effect in diabetic gastroenteropathy.

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Diabetic autonomic neuropathy and changes in oesophageal and duodenal contractility: neuronal and structural abnormalities

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Background and Aims: The pathogenesis of gastrointestinal symptoms in diabetes mellitus is complex in nature, multifactorial and not understood in detail. Diabetes induced neuronal changes are of major importance but also structural changes and remodeling of the gastrointestinal tract may be relevant.
Methods: The esophageal and duodenal activity to standardized bag distension was assessed in 12 diabetic patients with autonomic neuropathy and severe gastrointestinal symptoms and in 12 healthy controls using a specialized ultrasound based probe. the geometry and biomechanical parameters (strain, tension/stress, stiffness) were assessed.
Results: The diabetic patients had increased reactivity to esophagela distensions, e. g. increased frequency og distension-induced contractions ($p<0.001$). Impaired coordination of the contractions (dysfunction of local reflex arcs) was verified as ambalance between the time needed to evoke the first contraction at the distension site and proximal to it ($p<0.025$). The duration of the disease was associated with the increased activity. The esophageal wall thickness including all layers except the muscle layer was increased ($p<0.001$), longitudinal shortening decreased ($p<0.001$), and the radial compressibility increased ($p<0.001$) in the diabetic patients. The esophageal and duodenal wall stiffness and circumferential deformation induced by bag distensions were not affected by diabetes.
Conclusion: The impaired contractile acitivity observed may reflect neuronal abnomalities due to autonomic neuropathy but structural changes and remodeling of the GI tract may also interfere could explain the gastrointestinal dysfunction and symptoms observed in diabetes. This may have impact on the future management of diabetic patients with gastrointestinal symptoms.

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Diabetic autonomic neuropathy: aspects of differentiated visceral hypersensititation

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Background: The pathogenesis of gastrointestinal symptoms in diabetes mellitus is complex in nature, multifactorial and is not well understood. Diabetes induced neuronal changes (peripheral and central) may be of importance.
Methods: The visceral sensitivity to mechanical, thermal and electrical in the esophagus and duodenum was assessed in 15 diabetes patients with proven autonomic neuropathy and severe gastrointestinal symptoms and in 12 healthy controls using a specialized multimodal ultrasonic testing device. The sensory response and the somatic referred pain areas were recorded.
Results: An overall hyposensitivity to the combination of all visceral stimulations was found in the duodenum (P=0.01), and in the esophagus and duodenum combined (P=0.02). The visceral hyposensitivity was accompanied by an increase in the somatic referred pain areas (P=0.04) indicating central neuronal changes. Subanalysis revealed hyposensitivity to mechanical stimulations in the esophagus (P=0.006) and duodenum (P hyperexcitability) may be involved in the pathogenesis of the often severe and neglected gastrointestinal symptoms in longstanding diabetes. This can be of importance since future targets in the treatment of gastrointestinal symptoms in diabetic patients with autonomic neuropathy could be modulation=0.002), and to thermal (P<0.001) and electrical (P=0.005) stimulations in the esophagus and duodenum combined.
Conclusion: This multimodal and multisegmental sensory testing approach indicates that complex neuronal (interaction between peripheral hypoalgesia and central excitability)may be involved in the pathogenesis of the often severe and neglected gastrointestinal symptoms in longstanding diabetes. These finding may be of importance since future targets in the treatment of gastrintestinal symptoms in patients with diabetic autonomic neuropathy could be modulation of the central nervous system by receptor specific drugs or gastric electrical stimulation.

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Glucose metabolism improvement by gastric electrical stimulation in morbidly obese type 2 diabetic patients - interim results

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Background and Aims: The stomach plays a key role in the regulation of nutrient intake and disposition through neural, hormonal and mechanical functions. Previous work suggests that enhancement of gastric contractions during meals can induce weight loss in morbidly obese subjects. The TANTALUS™ System (MetaCure N.V.), a minimally invasive, non-restrictive, non-malabsorptive and non-excitatory gastric stimulation modality, may achieve improved glucose homeostasis and weight loss in obese diabetic subjects.
Materials and Methods: In an ongoing study, 24 adult obese T2DM subjects with different degrees of glycemic control and varied treatments (combinations of oral agents alone or with various insulin preparations) were implanted laparoscopically with the system, comprised of three bipolar electrodes stitched onto the stomach, connected to an implantable device. The device automatically detects food intake and delivers electrical signals synchronized to the stomach's natural rhythm. Mean baseline BMI and HbA1c in the patients were 41.7±0.9 kg/m^2 and 8.0±0.2%.
Results: Patients who were receiving insulin therapy had inconsistent responses. In the sub group of 9 patients inadequately controlled (HbA1c in the range of 7%–9.5% at baseline) on oral anti-hyperglycemic agents only, mean HbA1c at 20 weeks post-operative was reduced from 8.21± 0.28% to 7.04±0.25% ($p<0.05$) and fasting glucose decreased from 197.3±16.0 to 142.3±11.6 mg/dl ($p<0.05$). Weight loss was 6.4±1.5% of the baseline body weight ($p<0.01$). No significant device related complications or side effects were observed in the 24 patients.
Conclusion: Twenty week data indicate that gastric electrical stimulation initiated upon food intake detection improves glycemic control in morbidly obese T2DM subjects who are inadequately controlled with conservative oral medication. Improved glycemic control was accompanied by significant weight loss. The TANTULUS System appears to be a promising implantable device for the treatment of the obese type 2 diabetic patient inadequately controlled on oral anti-hyperglycemic agents.
Supported by MetaCure N.V

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A subclinical enteropathy precedes the clinical onset of type 1 diabetes

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Background and Aims: A number of recent studies indicate the presence of several intestinal abnormalities both in experimental and human autoimmune diabetes. In the BB rat increased intestinal permeability, mucosal lymphocytic infiltration, altered expression of mucosal associated mucins and intestinal intraluminal zonulin up-regulation have been reported. In patients with type 1 diabetes, increased intestinal permeability, intestinal biopsy-documented signs of enhanced immune activation and ultra-structural changes have also been shown. In human type 1 diabetes it remains to be clarified whether these alterations are related to an underlying pathogenetic process or simply reflect alterations secondary to the associated metabolic abnormalities. In order to elucidate this issue, we investigated the intestinal permeability to sugars in subjects at the different stages of type 1 diabetes. The aim was to further characterize the intestinal dysfunction associated with human type 1 diabetes and clarify its natural history in parallel with that of the underlying autoimmune process.
Materials and Methods: We investigated the permeability of intestine to sugars (monosaccharide Mannitol and disaccharide Lactulose) in subjects at different stages of type 1 diabetes: pre-clinical (17 relatives with ≥2 autoantibodies, median age 22, range 8–42 yr), clinical onset (21 patients, median age 28, range 6–56 yr), long-standing (33 patients, median age 41, range 22–66 yr, median duration of disease 19 yr, range 4–40 yr) and healthy controls (32 subjects, median age 26, range 9–54 yr). All subjects underwent a lactulose-mannitol test, consisting in oral administration of the two sugars and measurement by capillary electrophoresis of their urine excretion during the following 5 hours. Alterations of intestinal integrity may result in reduced passage of small molecules as mannitol (reflecting a reduced area of integral mucosa), and/or increased uptake of large molecules as lactulose (reflecting a damaged barrier).
Results: Intestinal permeability to lactulose was uniformly increased in all groups with islet autoimmunity, including pre-clinical, new onset and long standing type 1 diabetes (all $P<0.002$ vs healthy controls). Intestinal permeability to mannitol was significantly decreased only in long standing type 1 diabetes ($P=0.0185$ vs healthy controls).
Conclusion: These data provide evidence for the presence of a subclinical enteropathy associated with type 1 diabetes. Hallmarks of this enteropathy are: increased intestinal permeability to macromolecules (lactulose), already detectable before clinical onset, most likely associated with the autoimmune process; reduced permeability to small molecules (mannitol), detectable only in long standing disease, possibly secondary to diabetes. These findings bring to attention the possible participation of the small intestine in the pathogenesis of type 1 diabetes.

PS 99

Diabetic foot

1122

Simple clinical characteristics can identify diabetic foot patients at risk of poor outcome: the Eurodiale experience

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Background and Aims: Comprehensive data on clinical characteristics and outcome in diabetic foot disease is scarce; most information comes from single centres and from short-term studies. The Eurodiale study group has brought together 14 European centres specialized in diabetic foot care; one of its aims is to study the outcome of diabetic foot ulceration in relation to the clinical characteristics at presentation.
Materials and Methods: Data were obtained on 1229 consecutive patients with diabetes and a new foot ulcer. Data on patient characteristics, including co-morbidity, as well as foot and ulcer characteristics were obtained using a standardised case record form. Foot disease was categorised in 4 stages according to the presence of peripheral arterial disease (PAD) and infection: A (PAD -/infection -); B (PAD-/infection+); C (PAD+/infection-); D (PAD+/infection+). The patients were followed up until final outcome

(healing, major amputation or death) or up to one year from study entry.

Results: Outcome data are available on 1053 patients (mean age 65±12 yrs). In the whole cohort 77% healed; major amputation was performed in 4% and 7% of the patients died. Final outcome according to disease stage is shown in the table below. There was a significant difference for each outcome in relation to stage at presentation ($p<0.05$). Patient outcome was also related to the presence of disabling co-morbidities (end-stage renal disease, heart disease NYHA III/IV, severe visual impairment, inability to stand or walk without help): in such patients with one or more of these co-morbidities healing was significantly lower at 69% vs. 81% in patients without co-morbidities; amputation was performed more frequently (6% vs. 3%) and death occurred in 12% vs. 5% (all $p<0.05$). Ulcer characteristics (size, depth and location) were also associated with poorer outcome.

Conclusion: In this large European cohort healing rate was higher than expected. However, this study has demonstrated that the risk of poor outcome can be identified by using simple clinical parameters, including PAD, infection, co-morbidities and ulcer characteristics. Awareness of the usefulness of these clinical indicators should be promoted amongst all health care professionals working in the field of diabetes.

Outcome according to disease stage

Stage	*Healing*	*Amputation*	*Death*
A (n=270)	**86%**	**2%**	**3%**
B (n=305)	**84%**	**1%**	**5%**
C (n=205)	**78%**	**3%**	**6%**
D (n=347)	**65%**	**8%**	**14%**

Supported by EC 5th Framework Programme

1123

The prevalence of foot ulcer risk factors in Egyptian diabetic patients

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Background and Aims: The effect of ethnicity on diabetic foot ulceration (DFU), neuropathy and peripheral arterial disease (PAD) remains controversial. Contradictory results about the prevalence of DFU and amputation had been especially reported in diabetic patients of African descent living in UK or USA. The large nature of Africa continent that include diverse populations together with the possible effect of environmental and cultural factors on racial differences, make it plausible to study African patients in their original countries. Egypt is geographically located in Africa, however, it is also considered as a Mediterranean country. Few studies had reported the prevalence of DFU as part of diabetes related complications but, to our knowledge, there has not been any previous survey on DFU risk factors in Egypt. Objective: To study the prevalence of DFU and their risk factors in a cohort of patients presented to outpatient diabetes clinic at Mansoura University Hospital.

Materials and Methods: One thousand two hundred and twenty diabetic patients were screened over one year for DFU, neuropathy, PAD, foot deformities, skin and nail abnormalities. Patients were also questioned about receiving any previous education about foot problems or foot care.

Results: The mean age of our patients was 50.5+10.9 years and 36.8% were male. The mean duration of diabetes was 7.9+5.9 years and mean BMI was 34.5+6.7. The prevalence of active foot ulceration or past history of DFU was 1.2% and 5.7% respectively; so, the overall DFU prevalence was 6.9%. 5.07 SW monofilament insensitivity was found in 113 patients (9.3%). Only 38 patients (3.1%) had absent foot pulses that proved by doppler ultrasound to have PAD. The prevalence of Hallux valgus, hammer toe and flat foot was 23.4, 10.2 and 2.1% respectively. Dry skin and calluses were recorded in 544 patients (44.6%) and 69 patients (5.7%) respectively. Tinea pedis was found in 532 patients (43.6%). Thick nails and badly cut nails were found in 215 (17.6%) and 376 (30.8%) respectively. The shoes were inappropriate in 751 patients (61.6%) and 1145 patients (93.8%) didn't receive any prior foot education.

Conclusion: Despite the low prevalence of insensate neuropathy and PAD in our diabetic patients the prevalence of DFU is still high. The lack of knowledge regarding diabetic foot problems, the inappropriate foot wears and the high prevalence of skin and nail pathology in Egypt could explain this paradox. It is suggested that regional differences in the risk factors for DFU should be considered when preventative strategies for DFU are planed. Education of patients and healthcare providers together with establishment of podiatric care may have better outcome in our country.

1124

Diabetic foot care in French hospitals between 1997 and 2003

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Background and Aims: There is a lack of any published data in France on the extent of hospital admissions for diabetic foot ulcerations, lower limb amputations and revascularizations. Yet these are important markers to evaluate the quality of foot care in our country and its advances.
Materials and Methods: We have used the national database of the French Hospital Information System (PMSI) for public and private hospitalizations from 1997 to 2003. The selection of the ICD-10 codes for diabetes and its complications was crossed with codes for foot ulceration, procedures of revascularization, lower limb amputations, operations on stumps and debridements of wounds.
Results: Between 1997 and 2003, the French Health Care has registered a growing number of new treated diabetic patients, aged 18 years or more, on a rythm of+9% per year, with a total of 1.967.000 subjects on the 01/01/04 (3.27% of the French population). During the same period, hospital admissions for adult diabetic patients increased by 5.6% per year. We observed a progression of admissions for diabetic foot ulcerations (age and gender-adjusted rates :+7.3% per year), with a sharp increase of "non surgical" hospitalizations (+15.9% per year), while surgical stays grew slower (+2.9% per year). Each year, diabetic foot lesions were the cause of 8% of all diabetic admissions and accounted for 16% of total hospitalization days. The procedures realized during the "surgical"stays were, depending on the year, debridement in 14 to 18% of cases, revascularization in 40 to 43%, amputation in 40 to 42%. Amputation level was limited to toes or foot in 2/3 of cases, while major amputations (leg or thigh) occurred in 1/3 of cases. There was a development of the day cases, specially for non surgical care or debridements, growing from 17% in 1997 to 42% in 2003. For the other surgical stays (> 24 hours), their duration shortened by 3 days in case of a revascularization, by 2 days when a minor amputation was performed, and increased by half a day after a major amputation. Mean length of stay decreased from 19.6 to 18.1 days. Hospital admission rate for 1000 diabetic persons grew from less than 13‰ for patients under 55 years to 30‰ for those above 84 years. In 2003, the 35.900 hospital admissions for diabetic foot wounds concerned 24.400 patients. Diabetic men were more often hospitalized than women (68%) and were younger (mean age of 66.6 vs 70.3). They were more often implicated in stays leading to a revascularization or a limited amputation (75%). More than 80% patients had only one hospitalization during the year, 12% needed two stays and 7% three or more stays. The more the stays were numerous, the more the frequency of amputation was high. When a revascularization was associated to an other procedure, it was a minor one in 80% of cases. A high amputation occured in spite of a revascularization in 20% of cases. Regional rates of hospitalization for diabetic foot were not related with the prevalence of diabetes. Regional variations in the incidence of diabetes-related amputations were noted, while revascularization rates were very similar between the different French regions.
Conclusion: In spite of the development of conservative surgery during the last years, more than 4 "surgical" admissions for diabetic foot on 10 still lead to a lower limb amputation in France. Amputation rate for men is still 3 times more than for women.

1125

Bad news? Many patients with diabetic foot disease in Europe present with ischaemia, infection, and serious co-morbidity

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Background and Aims: Foot ulcers in diabetic patients have a variable clinical presentation and prognosis which is related to differences in underlying pathology, such as neuropathy, peripheral arterial disease (PAD) and infection. Large clinical studies describing the typical clinical presentation of diabetic foot ulcers are limited. In this study we assessed the characteristics of diabetic patients with a foot ulcer who were treated in 14 European hospitals.
Materials and Methods: Data were obtained on 1229 consecutive patients with diabetes and a new foot ulcer. Data on patient characteristics, including co-morbidity, as well as foot and ulcer characteristics were obtained using a standardized case record form. Foot disease was categorised in 4 stages according to the presence of peripheral arterial disease (PAD) and infection.
Results: At study entry 27% percent of the patients were admitted. Disabling co-morbidity was present in 32%. PAD was diagnosed in 49%, infection in 58%. The majority of the ulcers were located on the non-plantar surface of the

foot (52%). The frequency of the 4 stages was: A) PAD -/infection-: 24%, B) PAD-/infection+: 27%, C) PAD+/infection-: 18%, D) PAD+/infection+: 31%. Patients in this latter group had a distinct profile: they were older (age > 70 years 56% in stage D vs. 22% in A), had more non-plantar ulcers (65 vs. 36%), more extensive tissue loss (deep ulcers 64 vs. 20%; size > 5 cm^2 20% vs. 4%) and more serious co-morbidity (38 vs. 23%), all $p<0.05$.

Conclusion: We conclude that the clinical presentation of patients with diabetic foot ulcers in Europe is more severe than previously reported, in view of the presence PAD and infection in one third of the patients. Non-plantar foot ulcers are more common than plantar ulcers, especially in patients with severe disease, and serious co-morbidity increases significantly with increasing severity of foot disease.

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1126

What happens to the initial bone marrow oedema in the natural history of Charcot osteoarthropathy?

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Background and Aims: The aim of the study was to monitor what happens to the bone marrow oedema that is seen on Magnetic Resonance Imaging (MRI) in the early stages of Charcot osteoarthropathy.

Materials and Methods: We studied 12 diabetic patients with Charcot osteoarthropathy presenting with a hot swollen foot, hot isotope bone scan and normal radiograph. Sites of increased radionuclide uptake were metatarsal-tarsal (9), metatarsal-phalangeal (2) and ankle (1). Mean age was 55±10.6 years (mean±SD) and mean duration of diabetes was 32±13.7 years. There were 8 females and 4 males; type 1 diabetes (8) and type 2 diabetes (4). MRI was performed at presentation and at a median time of follow-up of 6 months (range 3–25 months). Offloading consisted of total contact casting in 8 patients and Aircasts in 4.

Results: The patients were divided into 3 groups; group 1 consisted of 6 patients with bone marrow oedema alone. On follow-up, 3 of these patients showed resolution of bone marrow oedema. In the remaining 3, one progressed to subchondral cysts, one to subchondral fracture and one to bony erosion with final reduction of oedema in all 3.

Group 2 comprised 3 patients with bone marrow oedema already associated with subchondral fracture. On follow-up, all 3 patients showed reduction of oedema and healing of fractures.

Group 3 comprised 3 patients with bone marrow oedema already associated with subchondral cystic change. On follow-up, definite subchondral cysts were seen with resorption of surrounding bone marrow oedema.

On follow-up of all patients, radiographs showed no subluxation and there was no clinical deformity.

Conclusion: We conclude that in the Charcot foot presenting with no radiological changes, bone marrow oedema, seen as the first lesion on MRI, may resolve completely or lead to subchondral fracture, subchondral cysts or erosions with eventual reduction of marrow oedema. This study gives new information on the natural history of the Charcot foot.

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1127

Propolis, the anti-inflammatory bee hive protectant improves wound healing in experimental diabetes

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Background and Aims: Up to 25% of patients with diabetes will develop a foot ulcer at some stage, leading in a minority to amputation. Consequently, treatments aimed at improving diabetic ulcer healing are of great importance. The naturally derived anti-inflammatory agent, Propolis has not been investigated in diabetic wound healing. This study examined the effect of a single topical application of Propolis in a standardised rodent model of diabetic wound healing. The main end-points determined were epithelial closure rate, total wound volume, and inflammatory cell infiltration.

Materials and Methods: Diabetes was induced in Sprague Dawley rats (n=16) using streptozocin (i.p. 65 mg/kg). Animals were maintained on 2nd daily insulin (2–4 IU Protophane). After 6 weeks of diabetes, six full thickness skin wounds were created on the backs of diabetic and control rats (n=16), using a 6 mm biopsy punch. Three wounds in each animal were treated at the time of wounding with 20 μl of either Propolis, or sterile saline control. Epithelial closure was monitored by progressive calliper measurement of lesion diameter. At days 6 and 12 after wounding, animals were sacrificed and the skin was removed, fixed and embedded. Tissue was serially sectioned for analysis of wound volume by polarised light microscopy, and neutrophil and macrophage number (mean of 20 fields) identified by morphology following PAS staining. Data are expressed as mean±SD.

Results: Compared with controls, diabetes caused a mean 27% decrease in epithelial closure rate. The delay in wound closure at day 6 and day 12 (shown in the table) was reversed by topical Propolis. Wound volume did not differ across the groups and the impaired tissue macrophage infiltration in diabetic wounds was not altered by Propolis. In contrast, persistent neutrophil infiltration observed at day 12 in diabetic rats, was abolished by Propolis.

	Epithelial closure (mm/day)	Wound volume (mm^3)	Macrophage (number/field)	Neutrophil (number/field)
Control	0.37±0.05	3.22±0.13	1.49±0.09	0.22±0.19
Diabetic	0.27±0.05*	3.86±0.21	0.25±0.14*	1.33±0.81*
Control +Propolis	0.43±0.06	3.36±0.71	0.89±0.39	0.17±0.17
Diabetic +Propolis	0.34±0.06	3.98±1.25	0.33±0.25*	0.23±0.19

* $p<0.05$ different from untreated control values

Conclusion: This data indicates for the first time that a single application of Propolis has utility in accelerating ulcer healing rate in diabetes. As neutrophil infiltration is normalised, its mechanism may be through anti-inflammatory actions. This result and the established safety profile of Propolis provide a rationale for studying topical application of this agent in a clinical setting.

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1128

Prospective randomized controlled study of non-ischaemic diabetic foot ulcers dressed with Hydrofiber® containing ionic silver or calcium alginate dressings

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Background and Aims: Diabetic foot ulcers (DFU) risk infection and impaired healing, placing patients at risk of lower extremity amputation. Debridement and dressings are required for DFU care. Hydrofiber® dressings retain more absorbed wound fluid and bacteria than alginate dressings and with ionic silver, persistently kill local pathogens. A prospective, multi-center study compared clinical efficacy and safety of AQUACEL® Hydrofiber® dressings containing ionic silver (AQ*) to those of calcium alginate (CA**) dressings used within regimens to manage out-patients with Type 1 or 2 diabetes and neuropathic or neuro-ischaemic Wagner Grade 1 or 2 DFU.

Materials and Methods: Patients were randomly assigned to similar protocols including off-loading, AQ or CA primary dressings and secondary foam dressings for 8 weeks or until healing. Assignment was stratified by baseline antibiotic use (Antibiotic) or non-use for the study ulcer, reflecting baseline clinical ulcer-related infection or its risk. Primary efficacy measures were healing outcomes. Safety was measured as adverse events.

Results: AQ and CA groups were comparable at baseline. Both groups improved on all ulcer healing outcomes. The time to healing was 53 days for AQ ulcers and 58 days for CA ulcers. AQ ulcers reduced in depth nearly twice as much as CA ulcers did (2.5 mm versus 1.3 mm; p=0.04). AQ subjects also experienced more overall ulcer improvement and less deterioration (p=0.06), accentuated in the Antibiotic subset (p=0.02). The safety profiles of both groups were similar.

Conclusion: A regimen including AQ significantly outperformed one using CA on ulcer depth reduction. This study reports the first significant clinical effects of a primary wound dressing containing silver on DFU healing.

Product Notations:
*AQ, AQUACEL® Hydrofiber® dressing with 1.2% ionic silver (AQUACEL and Hydrofiber are registered trademarks of E. R. Squibb & Sons, L.L.C., Princeton, NJ, USA)
**CA, Algosterile™ calcium alginate dressing (Algosterile is a trademark of Les Laboratoires Brothier, S.A., Paris-Nanterre, France); DFU, Diabetic foot ulcers

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The association of resistant staphylococcus aureus and the rate of lower limb amputations in patients with the diabetic foot

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Background and Aims: The progression of infection may contribute to lower limb amputation (LLA) in risk patients with the diabetic foot. Microbial resistance complicates further outpatient treatment of diabetic foot infection and may accelerate the progression of infection leading to amputations even if patients could be treated by i.v. antibiotics during hospitalization. The aim of our study was to assess the association of resistant Staphylococcus

Aureus (S.Aureus) and the rate of LLA in patients with the diabetic foot.

Methods: A total of 191 patients with diabetic foot ulcers and positive swab cultivation, whose were treated in our foot clinic from 12/1998 to 11/1999, were consecutively included into our study. The frequency of amputation was observed during a two-year period. Bacterial findings were established by cultivation of swabs taken from diabetic foot ulcers after local debridement. Microbial sensitivity to antibiotics was tested by sets by sets/using panels/batteries of antibiotics composed composed/developed on the basis of previous analysis of resistance at IKEM and by the recommendations of the National Reference Laboratory on Antibiotics. Microbial sensitivity to different types of antibiotics was determined semi- quantitatively by disc diffusion susceptibility10,(10,Courvalin, 191196; Reese, 1996). The occurrence of S.Aureus resistant to betalactam antibiotics was observed. Osteomyelitis (OM) was diagnosed by X-ray and laboratory markers of infection. Peripheral arterial disease (PAD) was detected by non-invasive methods (Doppler indexes<0.8, TcPO2 <30 mmHg) or angiography. Surgeons indicated LLA, if acute progression of infection or ischemia developed in the study patients.

Results: 50 patients (26%) underwent LLA (50/191; 44 patients had minor and 6 patients had major LLA) and 141 patients (74%) had no LLA during the observed period. 16.5% of all diabetic patients were shown to have resistant S.Aureus. Moreover, a higher occurrence of this pathogen was found in swabs of amputees before LLA compared with patients not undergoing LLA (33% and 11%); p<0.001. Based on Stepwise Logistic Regression, resistant S.Aureus (OR 4.34; CI 1.6–12.1), together with other factors such as OM (OR 15.4; CI 6.2–38.0) and PAD (OR 3.21; 1.1–9.2) were independent important risk factors associated with the rate of LLA. In addition, resistant S. Aureus was significantly associated with LLA especially in patients without OM (p=0.003; OR 7.1).

We **conclude** that not only OM and PAD but also the presence of S.Aureus resistant to betalactamsmicro may species could increase the incidence of LLA in patients with the diabetic foot, particularly those without OM.

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1130

Change in bacteriological analysis and in susceptibility to antimicrobial agents in diabetic foot infections

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Background: Infection is the primary problem requiring hospital admission of the patients with diabetic foot and it is the main reason of amputation. Aim of this study is to perform a bacteriologic analysis on lesions of diabetic outpatients with foot infections in order to characterize the pathogens more frequent, the variability of their prevalence in ten years (from 1995 to 2005) and to test the susceptibility to antimicrobial agents commonly used.

Methods: Were analyzed 100 cultures taken from the following sources: ulcers, abscesses, cutaneous bullae, bone or soft tissue specimens. They were placed in sterile transport containers Portagerm (Bio-Merieux) and promptly delivered to the clinical microbiology laboratory and were processed aerobically and anaerobically

Results: Staphyloccus aureus showed the highest prevalence (50%) and this was significantly increased if compared to the prevalence observed ten years before (20.6%). Pseudomonas aeruginosa showed the same trend with a prevalence of 23.2% against 8.7% at the first observation.The prevalence of streptococci show a not significant decrease (18.7% vs 21%). Furthermore we found a significant increase of polimicrobial infections (56.2 vs 48.6%) Regarding the susceptibility to the antimicrobial agents tested teicoplanin, vancomicin, linezolid and cotrimoxazol showed a 100% of susceptibility against staphyloccus aureus infection but teicoplanin had a lower MIC (<1,4). Ciprofloxacin with a percentage of 80% seems to be the most active against gram-negative. Imipenem showed the higher appearance of resistence, it was active only in 50% of infections

Conclusion: This study show that in 10 years there is a change in the prevalence of pathogens and in their susceptibility to antimicrobial agents with a significant increase of polymicrobial infections linked to the appearence of mulit-resistent pathogens as Staphyloccus aureus and Pseudomonas aeruginosa. Furthermore teicoplanin seems to be an anti-microbial agent of first choice in the treatment of Staphyloccus aureus infections.

PS 100

Glycation and advanced glycation endproducts

1131

Cellular events and signaling pathways activated by AGE-albumin interaction with the aortic endothelium

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Background and Aims: Diabetes is associated with increased formation and accumulation of advanced glycation end products (AGE), a heterogeneous group of compounds resulting from the non-enzymatic reaction between reducing sugars and proteins. Continuously exposed to high concentrations of both glucose and AGE-proteins, the diabetic vascular endothelial cells (EC) undergo structural, functional, and biochemical changes that eventually lead to a dysfunctional state. The aim of this study was to investigate (i) the interaction of circulating AGE-albumin (AGE-Alb) with aortic EC *in vivo* (binding, uptake, and degradation) and (ii) the signaling cascades activated upon AGE-Alb interaction with EC, *in vitro*.
Materials and Methods: The experimental approaches were: (i) streptozotocin-injected RAP mice perfused in situ with 70 μM AGE-Alb adsorbed to 5 nm colloidal gold (AGE-Alb.Au) followed by electron microscopy (EM); as controls, normal mice were employed; (ii) confluent human aortic EC cultured in DMEM supplemented with 25 mM glucose and exposed to 5, 15 and 30 μM AGE-alb for 5 to 60 min (controls: cells grown in 5.5 mM glucose and exposure to 5, 15 and 30 μM albumin). The expression of ERK1/2 (44 and 42 kDa MAPKs) was detected by immunoblotting using antibodies against the tyrosine-phosphorylated and total ERK1/2 proteins.
Results: EM studies showed that *in vivo*, AGE-Alb.Au bound to two EC transport structures: the caveolae and the coated pits. Binding to caveolae was followed by AGE-Alb.Au transcytosis, discharge and accumulation in the subendothelium; vascular infusion of anti-RAGE IgG diminished by ~50% the number of caveolae involved in AGE-Alb.Au uptake, indicating the role of caveolar receptors for AGE (RAGE) in the removal of circulating AGE-Alb. Upon interaction with the coated pits, the ligand was found in coated vesicles and multivesicular bodies, suggesting endocytosis and degradation of AGE-Alb. Although the EC pathways of AGE-Alb transport were identical in diabetic and normal animals, quantitatively, ~20% more caveolae transcytosed AGE-Alb.Au in diabetic aorta than in control, indicating enhanced permeability in diabetic conditions. In vitro assessment showed that human EC grown in 25 mM glucose and further subjected to 5 μM AGE-Alb exhibited a fast (up to 5 min) and significant increase (~4.3 fold) in ERK1/2 phosphorylation (as demonstrated by densitometry); concentrations of 15 and 30 μM AGE-Alb slightly augmented (~1.4 fold) ERK1/2 phosphorylation, as compared to control. A similar sequence of events but at lower amplitude took place at low (5.5 mM) glucose concentration: a rapid stimulation of ERK1/2 phosphorylation (~2.3 fold) at 5 μM AGE-Alb, and a nonsignificant enhancement (~1.2 fold) at both 15 and 30 μM AGE-Alb. While the expression of total ERK1/2 proteins in 25 mM glucose was ~1.6 fold above the control values (5.5 mM glucose) their concentration was not affected by the exposure to AGE-Alb. No changes in ERK1/2 phosphorylation were found in control samples challenged with 5–30 μM albumin.
Conclusion: The results indicate that in diabetes, AGE-Alb binds to EC-surface AGE-receptors that may be instrumental in the initiation of activation of ERK1/2. They also suggest that ERK1/2 and AGE-proteins contribute to EC activation and to the enhanced proliferative capacity in diabetic vascular endothelium.
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WITHDRAWN

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Influence of insulin resistance, pro- and anti-inflammatory markers, on soluble receptor for advanced glycation end-products (sRAGE)

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Background and Aims: In the development of diabetic micro- and macro-vascular complications, the interaction between advanced glycation end-products (AGEs) or ligands S100A12 with their receptors RAGE is thought to play a major role. RAGE has a circulating secretory receptor form, soluble RAGE (sRAGE), that neutralizing the action of its ligands might exert a protective role. The goal was to investigate if low sRAGE plasma levels were associated with insulin resistance and preferential abdominal fat accumulations, that are strong risk factors for the development of cardiovascular disease (CVD).
Materials and Methods: We studied 158 subjects, 83 with type 2 diabetes (T2DM) and 75 nondiabetic where we measured with ELISA systems sRAGE, *N*ε-(Carboxymethyl)lysine-protein adducts (CML), S100A12, adiponectin, C reactive protein (CRP).
Results: Plasma sRAGE was twice as low in T2DM subjects as in controls (140 [293] versus 735 [482] pg/mL, median [interquartile range], $p<0.0001$). Plasma sRAGE levels were inversely related to glycemic control, (as the fasting plasma glucose concentration, $r=-0.66$, $p<0.0001$), insulin resistance (as HOMA, $r=-0.39$, $p<0.0001$), levels of inflammatory markers (CRP, $r=-0.48$, $p<0.0001$), and

directly with adiponectin (r=0.25, p<0.008). Low sRAGE levels were also associated with preferential abdominal fat accumulation, as waist-to-hip ratio (r=−0.20 p<0.02) and visceral fat volume (VF, measured by MRI in a subgroup of subjects, n=84, r=−0.30, p<0.006), as well as with increased risk for CVD (Framingham score, r=−0.42 p<0.0001). On the other hand, while an inverse correlation was found between sRAGE and S100A12 (r=−0.64, p<0.0001), an important inflammatory molecule strongly connected to RAGE signaling, no association was found with advanced glycation end products such as CML (r=0.06, p=ns).

Conclusions: Low values of sRAGE are inversely associated with insulin resistance and pro-inflammatory markers, and directly with anti-inflammatory factors as adiponectin, indicating that this parameter is in first line to be used as a new marker in the predisposition to CVD, particularly in patients with T2DM.

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Increased plasma levels of sRAGE in patients with diabetic nephropathy

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Background and Aims: Advanced Glycation End products (AGEs) accumulate in diabetic patients in a greater rate than in age-matched non-diabetic subjects and exert various adverse effects via the Receptor of AGE (RAGE). Soluble RAGE (sRAGE) is a 50 kDa naturally occurring C-terminally truncated form of RAGE that functions as an inhibitor of AGE-mediated signaling events. Decreased levels of supposedly protective sRAGE were ascertained in patients with macrovascular disease and hypertension. Data about the role of sRAGE in diabetic nephropathy are so far lacking. The aim of the study was to analyse the relationship between sRAGE and renal functions in subjects with diabetic nephropathy and, hereby, implicit protective capacity against AGE-mediated toxicity.

Materials and Methods: Study was designed as a cross-sectional analysis of a cohort of 255 diabetic patients with variable extent of diabetic nephropathy. Total number of subjects consisted if 36 diabetics with T1DM, 219 with T2DM (approx. 1:1 male-to-female ratio). Mean duration of diabetes was 12.2±9.4 yrs, mean age was 61.3±14.5 yrs. Among all study subjects, 82 subjects had normoalbuminuria, 50 had persistent microalbuminuria, 112 had persistent proteinuria and 10 subjects had ESRD. Plasma concentration of sRAGE was assessed immunochemically (ELISA) using standard kits (Quantikine, RD Systems). Routine biochemical parameters were measured by certified methods using automated analysers. Relationship between circulating levels of sRAGE and the grade of diabetic nephropathy, renal function parameters and diabetes duration and compensation was analysed by means of non-parametric correlation, difference between groups were tested by Mann-Whitney test.

Results: sRAGE was significantly higher in nephropathy subjects (any degree of DN) than normoalbuminuric patients (P=0.0015), 2154.4±2612 pg/ml vs. 1622.7± 1981.4 pg/ml, respectively. In patients with nephropathy, sRAGE positively correlated with severity of nephropathy (P=0.0028). Further, sRAGE positively correlated with patient's age, duration of diabetes, serum urea, serum creatinine and albumin excretion rate and negatively with glomerular filtration rate (all P<0.05). No significant correlation was ascertained with fasting plasma glucose or HbA_{1c}.

Conclusion: Contrary to our anticipations, serum sRAGE levels are in fact (i) increased in diabetics with diabetic nephropathy compared to normoalbuminuric subjects and (ii) further rise with decreasing renal function. It remains to be elucidated whether the increase merely reflects decreased renal clearance of sRAGE or up-regulation of sRAGE in order to protect cells against AGE-mediated effects.

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Effect of AGEs on the expression of fractalkine in cultured human renal mesangial cells

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Background and Aims: Fractalkine (FKN, CX3CL1)is a membrane-bound chemokine which are proinflammatory cytokines due to its ability to activate endothelial cells and promotes strong chemoattractant and adhesion in glomerular diseases. Our previous studies showed that advanced glycosylation end products (AGEs) induced the expression of MCP-1 and RANTES, which belong to CC chemokine family members. The aim of this study was to characterize the effect of AGEs on the expression of fractalkine in cultured human renal mesangial cells (HRMC).

Materials and Methods: HRMC were exposed to vehicle (control), BSA, AGE-BSA (50 to 400 mg·L^{-1}) for 24 h, in turn, HRMC were stimulated with AGE-BSA(200 mg·L^{-1}) for various lengths of time (8 h,16 h, 24 h, 48 h, 72 h). Fractalkine mRNA and protein expression in HRMC were analyzed by reverse transcription-polymerase chain reaction (RT-PCR) and Western blotting.
Results: HRMC were incubated with different concentrations of AGE-BSA (50 to 400 mg·L^{-1}) for varying period (8 to 72 h). RT-PCR and Western blotting showed that basal state HRMC expressed a low level fractalkine mRNA and protein. AGE-BSA stimulated HRMC fractalkine mRNA and protein expression in a concentration-dependent manner, The maximal effect of AGE-BSA on HRMC fractalkine mRNA and protein expression was observed at 72 h.
Conclusion: AGEs may induce up-regulation of fractalkine on HRMC. These findings suggest that over-expression of fractalkine may be associated with inflammation in diabetic nephropathy, it might play an important role on progression of diabetic nephropathy.
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N$^{\varepsilon}$-(Carboxymethyl)lysine is increased in plasma and the kidney of spontaneously hypertensive rats and is reduced by losartan and spironolactone, but not by hydralazine

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Background and Aims: Pulse pressure is positively associated with risk of incident cardiovascular disease. We recently demonstrated that pulse pressure is strongly and independently associated with plasma levels of the advanced glycation endproduct, Nε-(Carboxymethyl)lysine (CML) in young type 1 diabetic individuals. Since the kidney plays a major role in the development of blood pressure we studied the accumulation of CML in the kidney during the development of hypertension in spontaneous hypertensive rats (SHR) and compared the effect of the antihypertensive specific angiotensin II receptor blocker losartan, the mineralocorticoid receptor antagonist spironolactone and the nonselective vasodilator hydralazine on the levels of CML in the kidney.
Materials and Methods: We determined plasma levels of CML in aged normotensive rats (n=8) and aged SHR rats (n=4) with tandem mass spectrometry. We analysed renal sections of very young (8 weeks of age) normotensive rats (WKY, n=6) and placebo- (n=6), losartan- (L: 20 mg/kg/day, n=6), spironolactone- (S: 1 mg/kg/day, n=6) and hydralazine-treated SHR (H: 15 mg/kg/day,n=6), which were subcutaneously treated for 4 weeks, for CML accumulation with immunohistochemistry and semiquantitatively analyzed. Mean arterial blood pressure was measured intra-arterially.
Results: SHR (2.32±0.69 μM) showed higher plasma levels of CML as compared to WKY rats (1.33±0.43 μM; $p<0.05$). Plasma levels of CML were correlated to pulse pressure in aged WKY rats and SHR (correlation 0.51; Rsquare 0.26). In SHR, all drugs lowered significantly blood pressure (L: 136±3, S: 136±2, H: 126±5 in comparison to placebo (SHR: 144±1 mmHg). In the kidney, CML staining was five-fold increased in proximal tubuli and as a thin endothelial cap in intrarenal arterioles in SHR (3.3±0.6AU), in comparison to WKY rats (0.7±0.5AU; $p<0.01$). As compared to untreated SHR, both losartan (1.2±0.6; $p<0.01$) and spironolactone (2.0±0.6; $p<0.05$), but not hydralazine (3.0±0.4), decreased CML staining in proximal tubuli and intrarenal arterioles in SHR.
Conclusion: The enhanced blood pressure increase in young SHR was associated with an increase of CML in renal parenchym and endothelial cells and may contribute to development and complications of hypertension. Despite similar blood pressure lowering, the effect of losartan and spironolactone on CML reduction indicates renoprotection of angiotensin inhibition beyond blood pressure lowering.
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Plasma levels of soluble receptor for advanced glycation endproducts (sRAGE) are associated with components of the metabolic syndrome: the CODAM study

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Background and Aims: The receptor for advanced glycation end products (RAGE) has been implicated in diabetes, inflammation and vascular disease. Soluble (s) RAGE is a C-truncated secretory isoform of the RAGE, that may neutralize the AGEs-mediated damage by acting as a decoy. Recently, sRAGE has been identified as a potentially important factor in the metabolic syndrome and atherosclerosis. Therefore, we studied potential determinants of plasma sRAGE levels, with a focus on components

of the metabolic syndrome, in individuals without and with type 2 diabetes, the CODAM cohort.

Materials and Methods: Plasma levels of sRAGE were measured with a commercially available Elisa (Quantikine, R&D systems) in subjects with normal glucose tolerance (NGT, n=298), impaired glucose tolerance (IGT, n=129) and in type 2 diabetes (DM2, n=147). To evaluate the relation between the levels of sRAGE with components of the metabolic syndrome, linear regression analyses were performed.

Results: Individuals with IGT and DM2 showed lower plasma levels of sRAGE compared to NGT subjects (NGT: 1408±599 pg/ml, IGT 1286±608 pg/ml and DM2 1202± 598 pg/ml) after adjustment for age, gender and BMI (IGT vs NGT $p<0.05$, DM2 vs NGT $p<0.01$). Moreover, gender (standardised (st) beta 0.27, $p<0.001$), HDL-cholesterol (st beta −0.19, $p<0.001$), waist circumference (st beta −0.22, $p<0.001$), diastolic blood pressure (st beta 0.12, $p<0.05$) and fasting glucose (st beta −0.12, $p<0.01$) were identified as independent factors associated with plasma sRAGE levels ($R^2=0.15$, $p<0.001$) in linear regression analysis studying components related to metabolic syndrome (triglycerides, systolic blood pressure and age: not significant). Since waist was one of the strongest predictors of sRAGE, we added the adipokines resistin, adiponectin and leptin to the above described model. This improved the model ($R^2=0.215$, $p<0.001$, p (F change)<0.001*)* with significant independent contributions of HDL cholesterol (st beta −0.21, $p<0.001$), waist circumference (st beta −0.23, $P<0.001$), gender (st beta 0.13, $p<0.05$), adiponectin (st beta 0.22, $p<0.001$) and resistin (st beta 0.09, $p<0.05$), but not of age, fasting glucose, triglycerides, diastolic and systolic blood pressure, and leptin.

Conclusion: Plasma sRAGE levels are decreased in IGT and DM2 subjects and are strongly related to components of the metabolic syndrome, especially adipocyte function. These data suggest that the sRAGE - RAGE axis is involved in components of the metabolic syndrome and its complications.

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PS 101

Endothelium, oxidative stress and inflammation

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Diabetes-induced effects on endothelial function and the actions of nitric oxide

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Background and Aims: Endothelial dysfunction with decreased NO (nitric oxide) bioavailability has been well documented *in vivo* in patients with both Type 1 and Type 2 diabetes. Impaired endothelium-dependent relaxation precedes both micro- and macrovascular complications. The aim of this study was to examine the endothelial function at two different stages (16 and 32 weeks) of diabetes in streptozotozin-induced diabetic rats, and to test whether diabetes induces changes in NO production or in the levels of oxidative stress or circulating advanced glycation end-products. We also examined whether similar changes in NO synthase (NOS) activity or NO production occur *in vitro* in endothelial cells when exposed to either normoglycemic (NG) and hyperglycaemic (HG) conditions.

Materials and Methods: Endothelium-dependent and independent vasorelaxation in the rat aortic rings were determined by constructing concentration-response curves for acetylcholine (Ach) and sodium niroprusside (SNP). Plasma levels of arginine metabolites were determined by high-performance liquid chromatography (HPLC). Carboxymethyl lysine (CML) was determined in plasma, and levels of oxidative stress in aortic tissue. The specific uptake of radiolabeled L-arginine and NO production were measured in bovine aortic endothelial cells (BAECs) after 7-day treatment in NG or HG environment.

Results: At 16 weeks of diabetes, the diabetic rats presented significantly delayed vasodilatory response to Ach as compared to the non-diabetic control rats (Ach EC_{50} −6.95±0.12 vs −7.37±0.20, $P=0.048$). At 32 weeks, the Ach curve was shifted to the right also in control animals and there were no significant between-group differences. Endothelium-independent vasodilatory responses to SNP were identical between controls and diabetics and showed no age-dependent change. The diabetic rats had significantly increased aortic intima-media thickness (IMT) at 16 weeks as compared to non-diabetic animals (0.95± 0.02 μm vs 0.83±0.01 μm, $P<0.001$). Aortic superoxide production was increased in diabetic rats at 32 weeks (914± 274 vs 235±37 U/5 mg of tissue, $P=0.034$ in diabetics vs controls, respectively), and plasma CML at 16 weeks of diabetes (705±114 vs 305±26 μmol/mol lysine, $P=0.018$). The diabetic animals had significantly decreased plasma levels of NO precursor L-arginine and of NG-hydroxy-L-arginine (NOHA), indicating inactive NOS function and decreased NO synthesis. They had increased levels of citrulline and asymmetric NG,NG-dimethyl-L-arginine (ADMA), an endogenous inhibitor of NOS. In vitro, similar basal levels of NO production and L-arginine uptake were observed in the cells in both HG and NG conditions.

However, insulin induced a significant increase in NO production in the HG-treated cells, suggesting an increased ATP-availability for the citrulline-to-arginine conversion in the HG environment *in vitro*.

Conclusion: Our results indicate decreased NO bioavailability in diabetic rats, potentially due to a blockade of the citrulline-arginine cycle. These results are in line with the observed decrease in the endothelium-dependent vasodilatation in these animals.

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A new mechanism linking IAPP and high blood pressure

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Background and Aims: IAPP has been involved in facilitating the development of High Blood Pressure (HBP), that is a frequent finding in patients with type 2 diabetes. There is very few data about the potential mechanisms by which IAPP could evoke an increase in HPB, being the stimulation of renin-angiotensin system the most known, although some data raise the possibility of the existence of other mechanism(s) in the development of HBP due to IAPP. The aim of this study was to test the interference with endothelium-dependent responses as an added potential mechanism for IAPP induced HBP.

Material and Methods: 15 week-old non-diabetic male Sprague-Dawley rats (400–450 g) were used. Rats were anaesthetised with 70 mg. kg-1 i.p. sodium pentobarbital and killed by bleeding. Aorta and mesentery were removed and placed in Krebs-Henseleit solution (KHS). For isometric tension recording both aortic segments (4–5 mm length) and segments of third branch mesenteric arteries (2 mm length; mean internal diameter range between 200 and 400 μm) were mounted in an organ bath and on a small vessel myograph respectively according to a method described previously.

Relaxation to ACh were subsequently assessed in both experimental approaches by adding increasing concentrations of the drug (10 nmol/L to 10 μmol/L in aorta; 1 nmol/L to 10 μmol/L in mesenteric arteries). In other set of experiments the contractile responses to noradrenaline (NA) were evaluated by adding cumulative concentrations to the organ bath (1 nmol/L-10 μmol/L in aorta; 10 nmol/L −30 μmol/l in mesenteric arteries). In both set of experiments, in some cases, the curves were performed after incubating some segments for 20 minutes with IAPP (5 or 20 pmol/L) and/or 100 μmol/l L NG-nitro-L-arginine methyl ester (L-NAME).

Results: In both aortic and mesenteric segments the preincubation with 5 and 20 pmol/l of IAPP did not change the maximal responses to NA. Otherwise the responses induced by ACh were significantly impaired by the preincubation with IAPP (20 pmol/L) but not with 5 pmol/L. L-NAME preincubation totally abolished the relaxant responses induced by ACh in aortic rings, and the presence of L-NAME plus IAPP (20 pmol/L) did not add any effect to the effect observed when segments were preincubated with L-NAME alone. In contrast to that observed in aorta, in microvessels L-NAME alone did not totally abolish relaxant responses to ACh but impaired them in a significant way, very close to the impairment produced by 20 pmol/L of IAPP. Otherwise no increased inhibitory effect on vascular relaxation was observed in the presence of L-NAME plus IAPP (20 pmol/L) in mesenteric microvessels.

Conclusions: We describe a new mechanism to explain the association between IAPP and HBP. Our findings support a direct effect of IAPP on vascular responses in macrovessels (aorta) and microvessels as well. This effect seems to be mediated by the interference of IAPP with the nitric oxide released by the endothelium.

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The role of oxidative stress in development of vascular insulin resistance in OLETF Rat, a model of type 2 diabetes

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Background and Aims: Reactive oxygen species was shown to induce vascular insulin resistance which is one of major risk factors in the development of vascular complications in diabetes, raising the possible involvement of alteration in insulin receptor substrate (IRS) proteins. This study was conducted to assess whether oxidative stress induced IRS protein degradation and enhanced serine phosphorylation, and to assess their functional importance in OLETF rats, an animal model of type 2 diabetes.

Materials and Methods: We used aortas of OLETF rats and LETO rats as control to measure IRS-1 protein expression, its serine phosphorylation and downstream metabolic insulin effects were measured.
Results: Superoxide production was markedly increased in aortas of OLETF rats compared to LETO rats. Furthermore, aortas of OLETF rats contained significantly less total glutathione and more 8-OHdG and nitrotyrosine, in comparison to controls. Aortas of OLETF rats exhibited decreased IRS-1 protein (but not IRS-1 mRNA and IRS-2 protein) expression, and increased phosphorylation of IRS-1 at Ser 307 compared with LETO rats. Total protein ubiquitination was also increased in aortas of OLETF rats. In contrast, there were no significant differences in expressions of insulin receptor, PI3K, PDK, and Akt/PKB between two groups.
Conclusion: These findings suggest that oxidative stress induces insulin resistance in the vasculature of OLETF rats specifically through increased serine phosphorylation of IRS-1 and its degradation by a proteasome-dependent pathway, providing an alternative mechanism that may explain the association with hyperinsulinemia with diabetic vascular complications

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HOMA IR and oxidative stress in non-diabetics

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Background and Aims: Oxidative stress has been proposed as on of the key factors in pathogenesis of insulin resistance and type 2 diabetes mellitus. The aim of our study was to determine whether markers of oxidative stress are elevated in relation to higher HOMA IR already in healthy nondiabetics.
Materials and Methods: We examined 67 men (aged 30–60 yrs.) with no history of atherosclerotic diseases (coronary heart disease, peripheral vascular disease, cerebrovascular stroke), no treatment for hypertension, heperlipoproteinemia or diabetes and with morning fasting venous plasma glucose below 6.0 mmol/l. The cohort was divided into tertiles of HOMA IR (calculated from fasting glycemia and insulinemia). In subsequent analysis we compared groups from the lowest (n=23, 47.99±9.051 yrs.) and highest (n=23, 51.00±7.472 yrs.; P=0.2220) tertiles. All individuals underwent physical and anthropometric examination, sonographic measurement of intima media thickness on common carotid artery (IMT) and endothelium dependent vasodilation on brachial artery (EDV). Morning fasting venous blood was collected for biochemical examination which included oxidized LDL (oxLDL), antibodies to oxLDL (aoxLDL), advanced products of protein oxidation (AOPP), lipid hydroperoxides (HPL), levels of retinol, tocopherol and total plasma antioxidation capacity (TAC). Concentrations of v.Wilebrand factor (vWF) and nitrites/nitrates were used as plasma markers of endothelial dysfunction.
Results: Subjects in the highest tertile of HOMA IR showed significantly higher levels of oxLDL (49.09± 8.051 vs 60.39±7.308 mU/l; P<0.05), AOPP (78.52± 17.118 vs 119.67±32.671 μmol/l; P<0.05) but surprisingly also of tocopherol (16.56±2.02 vs 19.54± 1.876 mg/l; P<0.05) although the TAC was not significantly different between the compared groups (0.12±0.013 vs 0.12±0.011 mmol/l; NS). Comparison of the highest vs lowest tertile groups revealed no significant difference in IMT (0.57± 0.119 vs 0.61±0.144 mm; NS), EDV (3.82± 2.051 vs 2.99± 1.516%; NS) or vWF (121.59±38.890 vs 141.53± 66.083%; NS).
Conclusion: Higher HOMA IR is accompanied by elevation of certain oxidative stress parameters already in asymptomatic healthy nondiabetic men even before manifestation of endothelial dysfunction.
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LPS as a mediator of sub-clinical inflammation in obese children and its' association with vascular injury and atherogenesis biomarkers

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Background: Obesity and its' later progression to Type 2 Diabetes Mellitus (T2DM) are both known to be associated with sub-clinical inflammation; although the underlying cause for this is unclear. However, studies suggest that bacterial endotoxin (LPS) derived from commensal bacteria in the human gastrointestinal tract may contribute directly to sub-clinical inflammation. Hence, with increasing adiposity the inflammatory response is exacerbated producing inflammatory adipocytokines, such as plasminogen activator inhibitor type-1 (PAI-1), IL-6 and TNF-α. This study therefore investigated the pathogenesis of the metabolic syndrome in obese children and its' association with inflammation, with LERC approval. Therefore, for this study, we examined the role of endotoxaemia in childhood

obesity through the application of multiplex cardiovascular disease (CVD) biomarker immunoassays to investigate the levels of a range of inflammatory and CVD risk markers.
Methods and Materials: Fasted serum was obtained from children with varying degrees of obesity (age±SD:13.9± 2.3 yr; BMI±SD:35.1±5.2 Kg/m^2; n=60). All children underwent an OGTT and insulin resistance was measured by homeostasis model assessment (HOMA-IR) and insulin secretion by Stumvoll index (ISI). The vascular injury markers for these obese children were measured using a multiplex assay system that analysed interleukin - (IL-) 1β, 6, 8 and 10, interferon-γ (IFN-γ), TNF-α, monocyte chemotatic protein-1 (MCP-1), n-terminal brain natriuretic peptide (NT-proBNP), vascular endothelial growth factor (VEGF), plasminogen activator inhibitor 1 (PAI-1), C-reactive protein (CRP), fibrinogen, serum amyloid A (SAA), serum amyloid P component (SAP), E-selectin, soluble intercellular adhesion molecule type 1 (sICAM-1), soluble vascular cell adhesion molecule type 1 (sVCAM-1), matrix metalloproteinase −9 (MMP9), myeloperoxidase lipid peroxide modified protein (MPO) and adiponectin.
Results: BMI correlated with several of the inflammatory markers (TNF-α, r=0.331*; CRP, r=0.310*; fibrinogen, r=0.291*; MPO, r=0.348**, *p<0.05, **p<0.01). However, endotoxin levels demonstrated a significant and positive correlation with the majority of markers for vascular injury and atherogenesis (TNF-α, myeloperoxidase; p<0.05; PAI-1, matrix metalloproteinase-9, monocyte chemotactic protein-1, soluble intercellular adhesion molecule type-1, vascular endothelial growth factor; p<0.01). These relationships remained significant following adjustment for sex, BMI and HOMA-IR.
Conclusions: In summary, these data highlight that subclinical inflammation in childhood obesity and T2DM may be mediated by circulating endotoxin levels. Furthermore, that children as young as 11 exhibit the same inflammatory profiles as identified in obese adults and may, as a result of long-term sub-clinical inflammation, have increased risk in the pathogenesis of T2DM and CVD at a much earlier age.
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Association between markers of subclinical inflammation, oxidative stress, endothelial dysfunction and metabolic parameters in impaired glucose regulation

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Background and Aims: The aim of our study was to evaluate the associations between markers of subclinical inflammation and endothelial dysfunction - indicative of increased cardiovascular risk-, metabolic parameters and markers of oxidative stress in people with impaired glucose regulation (IGR).
Materials and Methods: The study included 26 subjects with IGR (15 with impaired glucose tolerance [IGT]/11 with impaired fasting glycaemia [IFG], age: IGT:53.06± 3.10, IFG:46.,72±4.07 years; body mass index [BMI]: IGT:29.24±1.17, IFG:26.20±1.12 kg/m2; x±SEM) and 15 matched control subjects (C: age:49.60±3.06 years; BMI:26.27±1.01 kg/m2). Collection of fasting blood sample was followed by a 75 g oral glucose tolerance test and a second blood sampling 1 hour post load. White blood cell (WBC) count and high sensitivity C-reactive protein (hsCRP, immunoturbidimetry) were measured as markers of subclinical inflammation. Thiobarbituric acid reactive substances (TBARS, spectophotometry) and nitrotyrosine (NT, ELISA) were determined as markers of oxidative stress. Total antioxidant status (TAS, spectrophotometry) was used to assess the activity of the antioxidant defence system. Endothelial function was evaluated by determination of endothelium dependent (flow mediated - FMV) and independent vasodilation of the brachial artery by high resolution ultrasound in the fasting state and one hour post load.
Results: In addition to fasting blood glucose significant difference was found between the 3 groups in waist circumference (C:85.53±3.37, IFG:88.82±3.99, IGT:99.73± 3.55 cm; p<0.05), waist/hip ratio (C:0.81±0.03, IFG:0.87± 0.02, IGT:0.92±0.02) and WBC count (C:6.13±0.23, IFG:7.03±0.46, IGT:7.90±0.37 G/l). There was a trend of increasing hsCRP concentrations through the groups (C:1.31±0.23, IFG:1.97±0.54, IGT:2.65±0.38 mg/l), but the difference did not reach the level of significance. Fasting FMV was in the normal range in all three groups. One hour following the glucose load significant differences could be observed in blood glucose (C:8.38±0.56, IFG:10.56±0.43, IGT:12.10±0.,44 mmol/l), TBARS (C:3.83±0.30, IFG:5.76±0.58, IGT:6.00±0.49 μmol/l), TAS (C:0.99±0.05, IFG:1.13±0.06, IGT:1.22±0.05 mmol/l) and NT (C:16.84±7.89, IFG:87.17±21.35, IGT:168.54.06± 39.55 nmol/l) with a significant decrease of FMV only in the IGT group (13.63±1.86 to 5.81±1.78%). Significant positive correlation was found between WBC and TBARS (r=0.61), and NT (r=0.53) concentrations. hsCRP showed a positive correlation with insulin (r=0.43) and with 2 hour post load glucose (r=0.45). Fasting TBARS was found to correlate with cholesterol (r=0.58) and triglyceride levels (r=0.47). Postload TBARS and glucose (r=0.54), and postload NT and glucose (r=0.47) concentrations positively correlated also. In the IGR subgroup (n=26) hsCRP inversely

correlated with HDL cholesterol (r=−0.44) and NT positively correlated with TBARS (r=0.47).
Conclusion: In IGR altered levels of non-classical cardiovascular risk factors and markers of oxidative stress and endothelial dysfunction can be observed that are associated with metabolic parameters. According to the examined parameters subjects with IGT represent a higher risk group.

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Urinary orosomucoid excretion is related to inflammation and signs of endothelial dysfunction in patients with type 2 diabetes

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Background and Aims: Increased levels of inflammatory proteins and pro-inflammatory cytokines are associated with increased cardiovascular risk. Endothelial dysfunction is related to the development of atherosclerosis and predicts cardiovascular mortality in patients with type 2 diabetes. We have previously shown that increased urinary orosomucoid excretion rate (UOER) is an independent predictor of cardiovascular mortality in patients with type 2 diabetes. The aim of the present study was to evaluate markers of inflammation and endothelial dysfunction in patients with type 2 diabetes and normal or increased UOER.
Materials and Methods: We studied 41 patients with type 2 diabetes (17 with normal UOER and 24 with increased UOER; cut-off value of UOER: 2.04 μg/min) and 21 healthy subjects. Urinary orosomucoid, S-Orosomucoid and S-C-Reactive protein (CRP) were measured by immunoturbidimetry. P-Interleukin 6 (IL-6), P-Tumor Necrosis Factor-alfa (TNF-α), P-tissue Plasminogen Activator (tPA), P-soluble Intercellular Adhesion Molecule-1 (sICAM) and P-soluble Vascular Cell Adhesion Molecule-1 (sVCAM) were measured by ELISA. Endothelial function was also evaluated by measurement of the diameter of the brachial artery by external ultrasound at baseline and after flow-mediated vasodilatation versus post-nitroglycerin (NTG) vasodilatation using sublingual nitroglycerin.
Results: Mean (SD) age was 55 (9.4) years. In the 2 diabetic patient groups mean (SD) HbA1c was 8.4 (1.7)% and median (IQR) duration of diabetes was 5.6 (3.3–8.1) years. Analysis of variance revealed that patients with diabetes had higher concentrations of S-Orosomucoid ($p<0.001$), S-CRP ($p<0.001$), P-IL-6 ($p<0.001$), P-tPA ($p<0.001$) and P-sICAM ($p<0.003$) compared with healthy controls. Patients with increased UOER had higher concentrations of S-Orosomucoid ($p<0.001$), S-CRP ($p=0.001$), P-IL-6 ($p<0.001$), P-tPA ($p<0.001$) and P-sICAM ($p<0.002$) compared with healthy controls and higher P-IL-6 ($p<0.04$) than patients with normal UOER. In multiple regression analysis UOER independently predicted increasing values of S-Orosomucoid (OR (95%CI) 1.52 (1.23–1.88)), S-CRP (1.66 (1.24–2.22)), P-sICAM (1.38 (1.09–1.76)) and P-tPA (1.75 (1.28–2.39)). The analyses were adjusted for age, sex, systolic blood pressure, body mass index, HbA1c, serum cholesterol and urinary albumin excretion. There were no differences in P-TNF-α, P-sVCAM, flow-mediated vasodilatation or post-NTG vasodilatation between the three subgroups.
Conclusion: We conclude that patients with increased UOER and type 2 diabetes have a sub-clinical increased level of chronic inflammation and signs of endothelial dysfunction compared with healthy controls. Furthermore UOER is independently related to increasing values of markers of inflammation. Thus the pathophysiological link between increased UOER and cardiovascular mortality may be explained by vascular inflammatory activity and endothelial cell dysfunction in patients with type 2 diabetes.
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Elevated systemic chemokine concentrations precede the incidence of coronary heart disease and type 2 diabetes: results from the MONICA/KORA Augsburg study, 1984–2002

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Background and Aims: There is evidence from clinical and preclinical studies that chemokines may be involved in leukocyte infiltration into adipose tissue and in the pathogenesis of coronary heart disease (CHD) and type 2 diabetes (T2D). The aim of this study was to test the hypothesis that elevations of systemic levels of the chemokines MCP-1/CCL2 (monocyte chemoattractant protein-1), IL-8/CXCL8 (interleukin-8) and IP-10/CXCL10 (interferon-γ-inducible protein-10) precede the incidence of CHD and T2D.

Materials and Methods: Baseline serum concentrations of MCP-1, IL-8 and IP-10 were measured using a Luminex assay in a case-cohort design based on data from 381 individuals with and 1977 individuals without incident CHD and 526 individuals with and 1695 individuals without incident T2D from the prospective, population-based MONICA/KORA Augsburg study (1984–2002). The mean follow-up time was 11 years.
Results: Baseline serum levels of MCP-1, IL-8 and IP-10 were higher in CHD and T2D cases compared to the respective non-cases ($P \leq 0.001$ for all chemokines). After multivariable adjustment for other risk factors, individuals with concentrations of all three chemokines above the median level (compared to those subjects with all concentrations ≤ median) had no significantly increased CHD risk (hazard ratio 1.32 (95% confidence interval 0.88–2.00)), whereas the risk of incident T2D was significantly elevated (HR 1.79 (95% CI 1.18–2.72)).
Conclusion: Increased serum concentrations of the chemokines MCP-1, IL-8 and IP-10 are associated with incident CHD and incident T2D. This association appears more pronounced for T2D, and our data do not support the hypothesis that these chemokines are also independent risk factors for incident CHD.
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P66shc and oxidative stress in the physiopathology of type 2 diabetes complications

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Background and Aims: Oxidative stress plays an important role in cardiovascular dysfunction and atherogenesis. This is of particular interest in diabetes, a condition characterized by oxidative stress and increased prevalence of cardiovascular disease. The major role played by p66shc in oxidative stress-related response has been established by the resistance to and reduction of oxidative stress and prolonged lifespan of p66shc knockout mice. Recently we have demonstrated for the first time in type 2 diabetic patients an increase of mononuclear cell p66shc gene expression compared to healthy controls. Our results in patients with type 2 diabetes joined with those described in literature obtained *in vitro* and in animal models suggest a possible involvement of p66shc in the cellular and molecular mechanisms responsible of the dangerous oxidative stress mediated cardiovascular complications in type 2 diabetes.
In this study we have evaluated in skin fibroblasts of type 2 diabetic patients compared with healthy controls the effect of p66shc gene silencing on the production of reactive oxygen species (ROS).
Materials and Methods: After incubation with insulin (100 nM), ROS production was determined in human fibroblasts obtained from skin biopsies with a fluorescent probe (TEMPO-9-AC). p66shc gene silencing was performed using siRNA methodology.
Results: Insulin induced ROS production was significantly higher in fibroblasts from type 2 diabetic patients than in those from healthy controls (25,95±9,97 vs 21,21±8,19 arbitrary unit, a.u., $p<0,02$). p66shc silencing didn't change significantly ROS production in healthy controls (20,30± 7,01 vs 21,21±8,19 a.u., n.s.), while it induced a significantly decrease of ROS production in type 2 diabetic patients (18,73±8,42 vs 25,95±9,95 a.u., $p<0,01$).
Conclusion: Our results not only confirm the presence of increased oxidative stress in patients with type 2 diabetes, but, also, demonstrate in these patients an essential role played by p66shc in its induction. Since p66shc silencing reduces insulin induced fibroblasts ROS production. This is the first demonstration of a direct involvement of p66shc in insulin induced oxidative stress in type 2 diabetes which could give useful information for a possible therapeutic target in diabetic complications.

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Short-term intravenous insulin infusion and endothelial function in poorly controlled type 2 diabetes patients

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Background and Aims: Type 2 diabetes mellitus is associated with endothelial dysfunction, which is particularly enhanced at the stage of poor glycemic metabolic control. The study aimed at assessment of the effect of short-term continuous intravenous insulin infusion (IVII) on endothelial function and insulin resistance-related markers.
Materials and Methods: The study group consisted of 22 type 2 diabetes patients (mean age 51.8±17.0 years, BMI 28.5±2.1 kg/m^2, mean HbA1c 10.6±1.8%, mean fasting plasma glucose [FPG] 196±34 mg/dl, mean diabetes duration 8.3±2.7 years). IVII consisted of basal infusion rate of mean 1.9±0.5 IU/h and three mealtime 90-min boluses (mean 14±3 IU per bolus). Mean IVII duration was 4±1.4 days. Fasting serum soluble vascular cell adhesion molecule (sVCAM), soluble intercellular adhesion molecule (sICAM), sE-selectin, free fatty acids (FFA), adipo-

nectin, tumor necrosis factor-α (TNF-α) were measured with ELISA method immediately prior to the beginning and after the completion of IVII.

Results: After IVII mean FPG was 121±17 mg/dl ($p<0.01$ vs baseline). Before and after IVII serum sVCAM concetrations were 695±127 and 685±101 ng/ml ($p>0.05$), sICAM 347±90 and 330±117 ng/ml ($p>0.05$), sE-selectin 75.4±50.7 and 64.9±49.5 ng/ml ($p>0.05$), FFA 0.69±0.48 and 0.53±0.27 mmol/l ($p>0.05$), adiponectin 1877±2357 and 1366±964 ng/ml ($p>0.05$), TNF-α 31.3±47.7 and 11.1±17.1 pg/ml ($p>0.05$), respectively.

Conclusion: Despite marked improvement of glycemic control short-term continuous IVII in type 2 diabetes does not significantly affect endothelial function or insulin resistance-related markers, although apparent tendency towards less impaired endothelial function was noted. Upon our results it may be suggested that achieving improvement of endothelial function in type 2 diabetes patients requires longer duration of hypoglycemic treatment than it is necessary to achieve good glycemic control. However, it may also be speculated that it is solely duration of good glycemic control and not insulin treatment itself which is essential for improving endothelial function.

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Pancreas, liver and metabolic syndrome

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Pancreatic fat content, measured by proton-MRS in type 2 diabetes is associated with β cell failure

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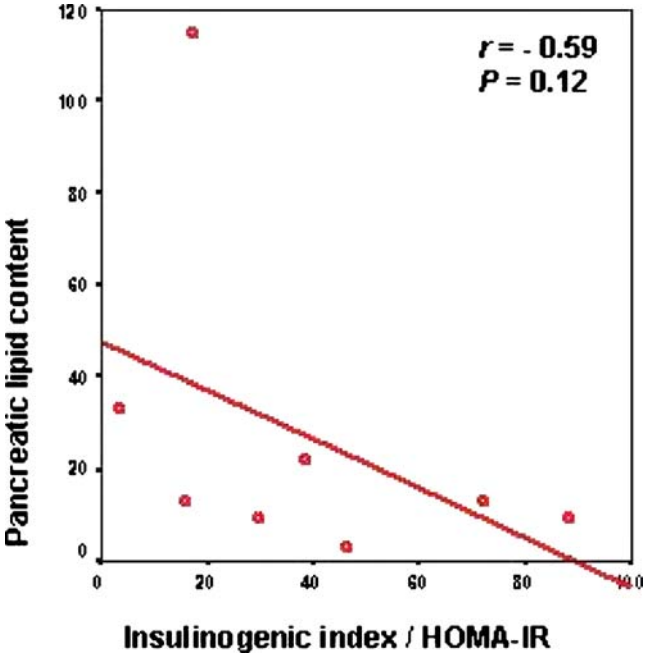
Background and Aims: Insulin resistance and central obesity lead to increased lipolysis with high flux of nonesterified fatty acids (NEFA) from adipose tissue and elevated hepatic production of triglyceride (TG)-rich particles. Due to the augmented lipid load in the presence of impaired glucose utilization, non-adipose tissues, including the pancreatic islets, accumulate TG. Excessive TG deposition in pancreatic islets enlarges the intracellular pool of fatty acyl-CoA, thereby providing substrate for non-oxidative metabolic pathways leading to oxidative stress and apoptosis, which, in susceptible subjects ultimately causes β-cell dysfunction (lipotoxicity). However, in human type 2 diabetes (DM2) pancreatic lipid accumulation *in vivo* and its association with β-cell function was never reported. Proton magnetic resonance (MR) spectroscopy may be used to quantify lipid content.

Materials and Methods: We measured pancreatic fat content in the fasting state by localized proton MR-spectroscopy in 4 DM2 (only oral blood-glucose-lowering therapy or diet, no TZDs or insulin) and 4 healthy age-matched males. Oblique voxels (10×10×20 mm) were placed in the pancreas, and 8 spectra were acquired and analysed separately. Occasionally, spectra showed dramatic increase of lipid signals, probably due to sudden deep breathing of the subject. These spectra were removed, and only spectra from voxels within the pancreas were subsequently used in the analysis of lipid versus water area. To estimate pancreatic β-cell function, we performed an oral glucose tolerance test (OGTT) and calculated insulinogenic index adjusted for insulin sensitivity (ΔI0–30/ΔG0–30/HOMA-IR).

Results: Patients (mean age 60 (SE 4.2) yrs; HbA1c 7.7 (0.8)%) versus healthy subjects did not differ in age, BMI, and waist. Mean pancreatic fat content was 42% in patients (range 9–115%) and 12% in controls (range 3–22%). β-cell function was impaired in patients (16.3 (5.4)) compared to controls (61.2 (11.5)). Pancreatic fat content was negatively associated with β-cell function ($r=-0.59$, $P=0.12$).

Conclusion: These preliminary results suggest that pancreatic fat content is elevated in subjects with DM2 relative to healthy controls and associated with decreased β-cell function. This finding may lead to future studies that should elucidate whether antisteatotic therapies targeting pancreatic fat accumulation indeed prevent DM2 in high-risk individuals.



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Screening for advanced fibrosis using non-invasive fibrosis biomarkers (FibroTest-FibroSure) in 789 diabetic patients

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Background and Aims: Type 2 diabetic patients (DI) vs non-DI have higher risk of non-alcoholic fatty liver disease (NAFLD) and liver cancer death. It is impossible to perform liver biopsy in such number of DI to identify advanced liver fibrosis (few septa F2, many septa F3, cirrhosis F4: F2F3F4). The aim was to identify F2F3F4 using non-invasive fibrosis biomarkers, **FibroTest (FT)**, previously validated in chronic hepatitis C, B, alcoholic and non-alcoholic steatosis.

Materials and Methods: Consecutive DI seen in a diabetes unit, (HCV HBV HIV neg), were prospectively included, as well as a prospective control group of blood donors (BD). Security algorithms permitted to exclude high-risk (HR) profiles of false negative/positive (HRFNFP)due to extreme values of components of **FibroTest** (alpha2macroglobin, haptoglobin, apolipoproteinA1, GGT, bilirubin). Analysis used univariate comparison with BD matched for gender, age, alcohol consumption and BMI, and multivariate logistic regression.

Results: From 1,994 subjects pre-included, 217 were excluded for HRFNFP, HCV, HBV, HIV and 1,777 were included: 519 type 2 DI, 237 type 1 DI, 33 other DI and 988 BD. 53%DI were male, mean age 55 yrs, 67% DI type 2; 83% had fasting glucose ≥6.1 mmol/L, 69% glycohaemoglobin ≥7.5%, 40% cholesterol ≥2.0 g/L, 27% triglycerides ≥1.5 g/L, 52% BMI ≥27, 47% arterial hypertension, 58% no alcohol consumption, 40%<50 g/d, and 2% >50 g/d. BD were younger (36 yr), had lower BMI (p<0.0001) and had more alcohol consumption. 239/584 (41%) DI had more than 30% steatosis detected by non-invasive biomarkers of steatosis (**SteatoTest**) versus 11/222 (5%) BD (p<0.0001). 41/584 (7%) DI had NASH (non-alcoholic steatohepatitis) detected by non-invasive biomarkers (**NashTest**) versus 0/222 BD (p=0.0006).

F2F3F4 were identified by **FibroTest** in 50/519 (9.1%) of type 2 DI, including 23 F2, 15 F3 and 12 F4, vs none F2F3F4 among the 988 BD (P<0.0001). 9.1% type 2 DI had F2F3F4 vs 0.8% DI type 1 (p<0.0001). Independent significant factors associated with F2F3F4 in multivariate regression analysis were type 2 DI (OR=5.4 p=0.03), AST ≥50 IU/l (OR=9.0 p=027), age ≥55 yrs (OR=6.6; p=0.009) and sex (OR=4.9; p=0.002).

71% F2F3F4 DI had steatosis >30% at **SteatoTest** versus 57% DI with minimal fibrosis (NS). 22% F2F3F4 DI had NASH by **NashTest** versus 9% DI with minimal fibrosis (p=0.01).

After exclusion of 170 not-matched type 2 DI, there were still 8.1% (28/349) F2F3F4 DI versus 0% BD (P<0.0001). 35/50 F2F3F4 DI, had ALT<50 IU/L, 18/50 GGT<50 IU/L, 16/50 both normal, 21/50 one of them and 13/50 both elevated. Only 2 DI had overt clinical-biological signs of cirrhosis attributable to an ancient heavy alcohol consumption.

Conclusion: The prevalence of advanced fibrosis (F2F3F4), estimated by **FibroTest**, is very high (9.1%) in type 2 DI followed in a tertiary center. This study strongly suggests that non-invasive biomarkers could be very useful for the screening of advanced fibrosis in type 2 DI to prevent liver mortality.

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Apolipoprotein-AII, insulinoresistance and steatosis in hepatitis C virus-infected patients

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Background and Aims: The mechanisms underlying steatosis during hepatitis C virus (HCV) infection are complex and multifactorial. A new pathophysiological mechanism of viral-induced liver steatosis was described for the core protein of HCV. It has been shown that the HCV-core protein reduces the activity of microsomal triglyceride transfer protein (MTP) and could lead to steatosis in HCV-infected patients. HCV-core protein markedly reduced hepatic microsomal triglyceride transfer protein activity, thereby interfering with VLDL assembly and secretion. Transgenic mice expressing HCV-core protein alone displayed decreased triglyceride secretion, whereas secretion was restored to normal in mice expressing both HCV-core protein and human apolipoprotein-AII (apo A-II). In consequence, it has been suggested that apo-AII could be a protective factor against HCV steatosis. One the other hand, it has been demonstrated that increasing plasma concentrations of mouse apoA-II in transgenic mice produced several aspects of the insulin resistance (IR) syndrome including hypertriglyceridemia, obesity, hyperleptinemia and IR. In the same way, some data suggested that the ApoA-II gene is implicated in visceral fat

accumulation and metabolism of triglyceride-rich lipoproteins. Both visceral obesity and insulin resistance are also implicated in the pathogenesis of HCV-steatosis. The aim of our study was to investigate the role of apo-AII in HCV-related steatosis.

Materials and Methods: Sixty-five consecutive untreated chronic HCV patients hospitalised for liver biopsy were included for this study to assess the effects of apo-AII, body mass index (BMI), age, insulin sensibility (HOMA), and leptin level on steatosis.

Results: Steatosis was observed in 36 (55.3%) patients. Apo-AII was significantly associated with HOMA (r=0.28; P=0.02) and with leptin concentrations (r=0.45; P=0.0002). In univariate analyses, age (46.6+/−11.5 vs 37.6+/−12.2, p=0.005), BMI (26.6+/−4.8 vs 22.8+/−2.8, p=0.001), increased leptin level (16.6+/−15.4 vs 11.1+/−11.3 μg/ml, p=0.05), increased HOMA (2.6+/−1.6 vs 1.4+/−0.8, p=0.001), and increased apo-AII concentration (0.42+/−0.07 vs 0.36+/−0.10, p=0.01) were associated with steatosis. In multivariate analysis, steatosis was significantly associated with apo-AII concentration, age, gender and BMI whereas steatosis was not associated with leptin, and HOMA.

Conclusion: At the opposite of previous hypothesis, Apo-AII is not a protective factor against HCV-steatosis but is significantly associated with the development of liver steatosis. The fact that the plasma levels of apo-AII correlate with HOMA and leptin levels in HCV-infected subjects suggests that apo-AII may contribute to hepatic steatosis progression probably in relationship to visceral obesity, IR or metabolism of triglyceride-rich lipoproteins. These preliminary data need further investigations.

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Endothelial function in non-diabetes subjects with non-alcoholic fatty liver disease

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Background and Aims: Non-alcoholic fatty liver disease (NAFLD) is associated with insulin resistance and glucose intolerance. However, whether NAFLD subjects are at increased risk of vascular injury is unclear. The study aim was to assess endothelial function in non-diabetes subjects with NAFLD.

Materials and Methods: Study group consisted of 30 subjects (mean age 38±9 years, body mass index 27.2± 3.2 kg/m2) with mildly elevated (up to 3 times over the upper reference limit) aspartate and alanine aminotransferases (AST and ALT) and 'bright' liver in ultrasound examination, without history of hepatitis viral infections, cardiovascular disease or diabetes. The controls were age-, gender- and body weight matched 20 subjects. All subjects had oral glucose tolerance test (OGTT) performed. Insulin resistance parameters including HOMA index were assessed and plasma endothelial function markers were measured in a fasting state.

Results: AST levels in the study group and controls were 76±22 and 23±9 IU/l (p<0.01), ALT 66±15 and 21±9 IU/l (p<0.01), respectively. In the study group and control subjects plasma glucose at 0, 60, 120 and 180 min OGTT were: 102±36, 171±53, 127±45, 88±32 and 82±24, 136± 42, 101± 22, 73±18 mg/dl (p<0.05), and plasma insulin 16.5±7.6, 93.0±56.7, 62.7±43.1, 21.5±17.8 and 7.1±3.2, 20.3±7.3, 15.3±7.7, 10.1±5.3 mIU/l (p<0.05), respectively. HOMA index was 4.7±3.5 and 1.4±0.4, respectively (p<0.01). Fasting plasma adiponectin in the study group and controls was 501±209 and 1450±423 ng/ml (p<0.01), tumor necrosis factor-α 18.7±23.9 and 9.3±8.7 pg/ml (p<0.01), free fatty acids 0.84±0.93 and 0.36±0.13 mmol/l (p<0.01), soluble vascular cell adhesion molecule (sVCAM) 2316±1198 and 1363±589 ng/ml (p<0.01), soluble intercellular adhesion molecule (sICAM) 559±290 and 202±78 ng/ml (p<0.01), sE-selectin 71.8± 26.5 and 27.4±5.3 ng/ml (p<0.01), respectively.

Conclusion: Individuals with NAFLD present with marked insulin resistance and mild glucose intolerance as well as signs of apparent endothelial dysfunction. NAFLD thus might be associated with increased risk of vascular injury; this risk is present before the development of overt diabetes.

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Lipoprotein particle distribution and size, insulin resistance and the metabolic syndrome in Alaska Eskimos. The GOCADAN Study

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Background and Aims: Insulin resistance is associated with a dyslipidemia characterized by increased VLDL,

lower HDL and a preponderance of small-dense LDL particles. The former two abnormalities are included in the definition of metabolic syndrome. Within each lipoprotein class there is a particle size and density heterogeneity, whose relationship with insulin resistance and metabolic syndrome has been only partly investigated. Recently, Nuclear Magnetic Resonance (NMR) provides an advanced, rapid and cost-effective method to measure lipoprotein distribution and size in large population samples. Therefore, the aim of this study was to determine how lipoprotein subclass distribution and size are related to insulin resistance and metabolic syndrome in a population based sample of Alaska Eskimos over a wide age range.

Materials and Methods: In 1121 adult (age ≥18 yrs) men and women, VLDL, LDL, HDL particles distribution and size were evaluated by NMR and related to the presence of metabolic syndrome (ATP III criteria) and to the level of insulin resistance (HOMA-IR index).

Results: Subjects with metabolic syndrome (n=157), compared to those without (n=964), were characterized, independently of gender, age, body mass index, C-reactive protein, current and ex smoking, by: 1) a significant, higher percentage of large/intermediate VLDLs (p=0.0001) and lower percentages of large/intermediate LDL (p=0.0001) and HDL particles (p=0.0001), 2) a significant, higher VLDL size (p=0.0001) and lower sizes of both LDL and HDL (both p=0.0001). Dividing the whole population according to tertiles of HOMA-IR, percentage of large/intermediate VLDL was significantly higher (p=0.0008 and 0.02, respectively) with increasing HOMA-IR tertile, while percentages of large/intermediate LDL and HDL particles were significantly lower (p=0.02 and 0.0009, respectively). VLDL size significantly increased with increasing of HOMA-IR tertile, while LDL and HDL sizes were significantly decreased (all p=0.0001).

Conclusion: In this population, both insulin resistance and metabolic syndrome are characterized by a complex spectrum of VLDL, LDL, and HDL distribution abnormalities that are independent of obesity, age and other CVD risk factors.

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Circulating $CD34^+$ cells, metabolic syndrome and cardiovascular risk

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Background and Aims: Cardiovascular diseases (CVD) are the leading cause of death in western countries and risk assessment has become mandatory in the adult population. Over the past decades, it has become clear that patients with a given combination of risk factors, clustered in the so-called metabolic syndrome (MetS), are more prone to develop CVD. Besides the classical risk factors, novel biomarkers have recently been proposed, among which, circulating progenitor cells (CPCs). CPCs are believed to participate in cardiovascular (CV) homeostasis and their exhaustion has been linked to CV damage. This work was carried out to assess the relationships between different antigenic profiles of progenitor cells and CV risk, with special regard to MetS.

Materials and Methods: Two hundred and fourteen subjects (56% male; age 56.4±14.9 years) with different levels of CV risk participated in the study. CV risk was defined considering: diabetes mellitus, obesity, hypertension, dyslipidemia, smoke, age >50 years. MetS components were assessed using ATP-III criteria. 10-year Framingham risk was assessed as well. Flow cytometric analysis was used to quantify six different CPC subtypes ($CD34^+$, $CD133^+$, $CD34^+CD133^+$, $CD34^+KDR^+$, $CD133^+KDR^+$ $CD34^+CD133^+KDR^+$).

Results: The study sample was representative of an intermediate-high CV risk (mean Framingham risk 18%). Among the six different progenitor cell subtypes, $CD34^+$ cells showed the best correlation with all CV parameters. Components of the MetS were all characterised by reduction of $CD34^+$ cells, and their clustering acted synergically in decreasing $CD34^+$ cell count. Linear correlations between $CD34^+$ cells and age ($r=-0.40$), blood glucose ($r=0.34$), systolic blood pressure ($r=-0.33$), LDL ($r=-0.22$), HDL ($r=0.23$), triglycerides ($r=-0.34$), and waist circumference ($r=-0.23$) were significant after adjustment for α-inflation. Significant correlation was also detected between $CD34^+$ cells and 10-year Framingham risk ($r=-0.40$), excluding subjects with established CVD, for whom the Framingham algorithm is not applicable. Consistently, a multiple logistic regression showed that $CD34^+$ cell count was an independent predictor of high (>20%) 10-year risk, suggesting that progenitor cell count may provide further information on cardiovascular risk beyond classical risk factors. Finally, a ROC curve analysis indicated a high performance of $CD34^+$ cell count in detecting high CV risk.

Conclusion: These data demonstrate that CD34 identifies CPC linked to CV risk, showing a close negative correlation between $CD34^+$ cells and CV risk as well as a synergic detrimental effect of clustered metabolic components. CPC count may be used as a surrogate marker of CV risk whereas extensive antigenic characterisation may not be useful for this purpose.

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Impacts of the metabolic syndrome and Framingham risk score on cardiovascular predictors in patients with type 2 diabetes

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Background and Aims: The metabolic syndrome (MetS) is a constellation of interrelated risk factors to promote the development of atherosclerotic cardiovascular disease (CVD). In type 2 diabetes mellitus (T2DM), however, it has not yet been clarified whether the identification of the MetS improves the prediction of cardiovascular (CV) events. Framingham risk score (FRS) is an established predicting model for CVD. We sought to compare the impact of MetS with FRS on CV predictors in patients with T2DM.
Materials and Methods: One hundred and seven patients with T2DM (50 males and 57 females) were enrolled. Patients with history of CVD, any inflammatory disease and anti-hyperlipidemic medication were excluded. MetS was defined by modified NCEP-ATP III criteria. High-sensitivity C-reactive protein (hsCRP), homocysteine, lipoprotein(a), fibrinogen, uric acid and γ-glutamy transferase were regarded as CV predictors.
Results: 71.4% of total patients had the MetS. The levels of all CV predictors was not different between diabetic patients with and without MetS. Any CV factors were also not significantly different between numbers (1, 2, 3, 4 and 5) of components of the MetS. However, homocysteine ($r=0.364$; $p<0.001$), fibrinogen ($r=0.202$; $p<0.05$) and uric acid ($r=0.298$; $p<0.05$) levels were positively correlated with FRS. Homocysteine (8.9±5.3, 9.7±2.7, 11.8±4.5, 12.8±6.1 and 12.5±3.2 μmol/L; $p<0.01$) and uric acid (4.1±2.2, 4.8±1.6, 4.9±1.5, 6.5±3.6 and 5.9±0.7 mg/dL; $p<0.05$) levels showed significant differences according to increasing quintiles of FRS.
Conclusion: The MetS is not related to CV predictors as well as FRS in patients with T2DM. It suggests that categorizing type 2 diabetic subjects as having or not having the MetS does not provide further prediction of CVD.

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The relationship of metabolic syndrome and its specific combinations with all cause mortality and coronary heart disease among type 2 diabetic patients

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Background and Aims: Although most studies investigating the relationship of metabolic syndrome (MBS) with all cause mortality or coronary heart disease (CHD) concern general population, there is lack of studies examining this relation among type 2 diabetic patients. The aim was to asses if MBS components or its several combinations have a higher predictive value than MBS alone in the evaluation of CHD and all cause mortality, among type 2 diabetic patients.
Materials and Methods: A total of 600 patients were enrolled in this analysis, from 1989 until 2004 with a mean follow up of ten years. In each patient the presence of MBS (NCEP ATP III), the cumulative number of MBS components, age, sex, blood pressure, HDL, LDL, total cholesterol, TG, HbA_{1C}, smoking status and diabetes duration were determined at baseline. Information regarding death or CHD events was obtained from our diabetic outpatient database and from hospital discharges. Statistical analyses were performed using Kaplan -Meier estimator and Cox proportional Hazard models.
Results: 62.4% had MBS. Significant predictors for all cause mortality were the presence of MBS (H.R: 1.75), sex (H.R: 1.68), age (H.R: 1.09), and diabetes duration (H.R: 1.02). With respect to CHD incidents, HDL (H.R: 0.97), systolic blood pressure (H.R: 1.012), and total cholesterol (H.R: 1.005) were significant predictors. MBS was not significant predictor in multivariate analysis after adjusting for CHD conventional risk factors. Subjects meeting the triad consisting of diabetes-hypertension- low HDL or the quadruple diabetes- hypertension- low HDL- high TG levels presented the higher probability for developing CHD events. (H.R: 1.79, 1.73 respectively).
Conclusion: The coexistence of MBS in type 2 diabetic patients decreases the survival probability. MS combinations mentioned above seems to confer higher risk regarding CHD prediction than other MS combinations or MS alone.

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Prospective evaluation of International Diabetes Federation 2005 and Adult Treatment Panel III 2001 criteria of the metabolic syndrome among women undergoing coronary angiography

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Background and Aims: The International Diabetes Federation (IDF) has recently established a worldwide consensus definition of the metabolic syndrome (MetS). The aim of this study was to prospectively evaluate the cardiovascular risk associated with the IDF and Adult Treatment Panel III (ATPIII) MetS definitions in women undergoing coronary angiography.

Materials and Methods: In a prospective study on 241 women undergoing coronary angiography for the evaluation of coronary artery disease we recorded vascular events over 4 years.

Results: From the women enrolled in our study, 34.9% (n=84) had the MetS according to the ATP III definition and 47.8% (n=115) according to the IDF definition. Concordance between the definitions was moderate (Cohen-Kappa coefficient=0.537; p<0.001). The MetS as defined by the ATP III criteria significantly predicted vascular events (adjusted hazard ratio=2.133 [1.111–4.094]; p=0.023) but the MetS as defined by IDF criteria did not (adjusted hazard ratio=1.214 [0.635–2.319]; p=0.558). Accordingly, event-free survival was significantly lower among women who fulfilled the ATP III but not the IDF criteria than among those who met the IDF but not the ATP III criteria (p=0.035).

Conclusion: Among women undergoing coronary angiography for the evaluation of CAD, the ATP III and the IDF definitions of the MetS do not identify the same patient populations. The ATP III definition of the MetS confers a significantly higher risk of vascular events than the IDF definition.

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Glitazones: mechanisms, effects and costs

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Rosiglitazone inhibits the upregulation of fractalkine induced by AGEs *in vivo* and *in vitro*

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Background and Aims: Our previous studies showed that advanced glycosylation end products (AGEs) induced the expression of CC chemokines such as MCP-1 and RANTES, and played an important role in the progression of diabetic nephropathy (DN). fractalkine (CX3CL1) is a unique membrane-bound CX3C chemokine that serves as a potent chemoattractant and adhesion of monocytes via its receptor CX3CR1, was observed upregulation by AGEs in cultured human renal mesangial cells (HRMC). This study was to investigate the influence of rosiglitazone on the up-expression of fractalkine by AGEs in HRMC. We also observed the effect of rosiglitazone on the fractalkine expression in rat renal cortex.

Materials and Methods: HRMC were incubated with AGE-bovine serum albumin (AGE-BSA)in presence or absence of rosiglitazone for 24 h. Type 2 diabetic rats were induced by an intraperitoneal injection of **low doses of** streptozotocin combined with **high fat diet**, and were randomized to receive rosiglitazone or vehicle treatment. Serum AGE-peptide was measured by flow injection assay. The mRNA and protein expression of fractalkine in HRMC were analyzed by semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) and Western blotting, respectively. The expression of fractalkine in renal cortex of rats was examined with RT-PCR and immunohistochemical staining, respectively. Data were expressed as the mean± standard deviation of at least three independent experiments. Comparisons among groups were done by one-way ANOVA.

Results: 1. After incubation of HRMCs with AGE-BSA for 24 hours, both mRNA and protein expression for fractalkine were significantly increased compared to corresponding BSA group. 2. The elevated expressions of fractalkine mRNA and protein were decreased by exposure to rosiglitazone in a concentration dependent manner. 3. The serum AGE-peptide, the level of fractalkine mRNA and protein in renal cortex of diabetic rats was significantly higher than those of normal control. However those of rats treated with rosiglitazone were obviously lower compared to untreated group.

Conclusion: Rosiglitazone can inhibit the upregulation of fractalkine by AGEs *in vivo* and *in vitro*. These results suggest rosiglitazone might play a role of reno-protection via supression of AGEs and fractalkine.

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Pioglitazone acutely influences glucose-sensitive insulin secretion in normal and diabetic human islets

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Background and Aims: Peroxisome proliferator-activated receptor-γ (PPARγ) plays an important role in the regulation of energy homeostasis. Pioglitazone is an orally administered insulin-sensitizing agent that activates PPARγ, leading to increased transcription of genes encoding various proteins regulating glucose and lipid metabolism. In contrast to its action on insulin target tissues, little is known about the direct action of the PPARγ agonist on pancreatic β-cell function. The aim of the study was to investigate acute effects of the PPARγ agonist pioglitazone *in vitro* on human islets from both non-diabetic and type 2 diabetic subjects.
Materials and Methods: Human islets from adult non-diabetic or type 2 diabetic donors were maintained in tissue culture medium in the presence of 5.5 mM glucose. Pancreatic islets from *ob/ob* mice were isolated by collagenase digestion. Insulin secretion dynamics from perifused human islets was monitored using micro-columns and factions were collected every two minutes. Insulin secretion from batch-incubated islets was evaluated in 24-well plates during 20 min-incubation. The insulin content in the fractions was measured using human or rat ELISA kits. Cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured in Fura-2 pre-loaded single islet cells prepared from human islets, using a time-sharing spectrofluorometer equipped with an inverted epifluorescence microscope.
Results: In 5 mM glucose, pioglitazone caused a slight and transient increase in insulin secretion in non-diabetic, but not diabetic, islets. Continuous presence of the drug suppressed insulin release in non-diabetic islets. In islets from non-diabetic subjects, both high glucose and tolbutamide-stimulated insulin secretion was inhibited by pioglitazone. When islets from either non-diabetic or diabetic subjects were perifused with 5 mM glucose, short-term pretreatment with pioglitazone caused approximately 2-fold increase in insulin secretion but only after drug withdrawal. Pioglitazone pre-treatment of diabetic islets restored their glucose sensitivity. Examination of cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) in non-diabetic islets revealed slight Ca^{2+} transient by pioglitazone at 3 mM glucose. In contrast, the suppressed insulin secretion of the drug at higher glucose concentrations was not associated with changes in $[Ca^{2+}]_i$.
Conclusions: Our data indicate bimodal acute effects of pioglitazone directly on human islets and suggest that short-term pretreatment with pioglitazone primes both healthy and diabetic human islets for enhanced glucose-sensitive insulin secretion. This mechanism might be involved in the β-cell sparing effects of pioglitazone and other thiazolidinedione PPARγ agonists.

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Pioglitazone treatment in patients with type 2 diabetes and a history of myocardial infarction: subgroup analyses from PROactive stratified by gender, age, and duration of diabetes

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Background and Aims: Compared with the general population, individuals with type 2 diabetes have an increased incidence of myocardial infarction (MI). PROactive studied the effects of pioglitazone on top of standard therapy on a composite macrovascular endpoint, which included non-fatal MI. This exploratory subanalysis of the larger PROactive study assessed 1,804 male and 641 female patients with a previous MI (≥6 months prior to randomisation).
Materials and Methods: PROactive was a double-blind, placebo-controlled study in 5,238 patients with type 2 diabetes and macrovascular disease randomised to pioglitazone or matching placebo in addition to existing glucose-lowering and cardiovascular medications, including antihypertensive, antiplatelet, and lipid-modifying drugs. The starting dose of pioglitazone was 15 mg/day, which was titrated to a dose of 45 mg/day, if tolerated. Mean follow-up was 34.5 months. Investigators were encouraged to treat diabetes, dyslipidaemia, and hypertension optimally according to the International Diabetes Federation guidelines. Analyses were performed according to gender, age (<65 years [n=1452], ≥65 years [n=993]), and duration of diabetes (<5 years [n=731], ≥5−<10 years [n=673], ≥10 years [n=1040]).
Results: The occurrence of a recurrent fatal or non-fatal MI (excluding silent MI) in patients with previous MI was

significantly lower in those treated with pioglitazone than in those treated with placebo (5.3% *versus* 7.2%, HR=0.72, P=0.045). Interaction tests showed that there was no significant impact of gender (P=0.53), age<65 years/ ≥65 years (P=0.43), or diabetes duration<5 years/ ≥5−<10 years/≥10 years (P=0.46) on the hazards ratio for time to fatal/non-fatal MI (excluding silent MI). This suggests that the best estimate for the reduction in risk of recurrent MI for each subgroup is given by that for the entire previous MI cohort. Median HbA_{1c} and triglycerides decreased and HDL-C increased to a greater extent in the pioglitazone group than in the placebo group in the total cohort of patients with previous MI and also in all of the gender, age, and diabetes duration subgroups (P<0.0001 for all comparisons except triglycerides in the ≥5−<10 years duration subgroup, P<0.03).

Conclusion: Pioglitazone has been shown to have beneficial effects on hyperglycemia as well as diabetic dyslipidemia and pleiotropic markers associated with macrovascular disease. In this exploratory subset, pioglitazone had a consistently better effect on recurrent MI and metabolic parameters than placebo did, irrespective of gender, age, and duration of diabetes.

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Glycaemic and lipid effects of pioglitazone and rosiglitazone in patients naive to oral antidiabetic medications

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Background and Aims: Therapeutic responses to oral antidiabetic medications (OAM), such as the thiazolidinediones (TZDs) pioglitazone (PIO) and rosiglitazone (ROSI), are affected by factors including baseline A1C values, duration of disease, and history of prior OAM. This subgroup analysis from the GLAI study evaluated the glycemic and lipid effects of PIO and ROSI in patients with type 2 diabetes (T2D) and no prior history of OAM.

Materials and Methods: GLAI was a prospective, randomized, double-blind, multicenter trial comparing glycemic and lipid effects of PIO and ROSI in patients with T2D (WHO criteria) and dyslipidemia (fasting TG levels ≥150 and <600 mg/dL; fasting LDL-C levels=130 mg/dL). A prespecified subgroup comprised 197 patients previously naïve to OAM (PIO, n=88; ROSI, n=89). Following randomization and a 4-week placebo run-in phase, patients received PIO (30 mg QD) or ROSI (2 mg BID). At 12 weeks PIO and ROSI were titrated to maximally effective doses of 45 mg QD and 4 mg BID, respectively, and maintained for an additional 12 weeks.

Results: Both treatment groups had similar A1C levels at baseline (Mean+SD, PIO: 7.99±0.12%, ROSI: 7.79± 0.12%). Comparable reductions from baseline in A1C were observed for both treatment arms (PIO: −1.13± 0.09%, P<0.05; ROSI: −1.05±0.09%, P<0.05) with no significant between-group difference (P=0.491). By study's end, 44/88 (50%) of PIO and 40/89 (45%) of ROSI patients reached the ADA target of A1C<7.0%. The serum lipoprotein results are shown below.

	Week 24 Changes from Baseline					
	PIO N=88			ROSI N=89		
	Baseline	Mean change ±SEM	% change	Baseline	Mean change ±SEM	% change
TG (mg/dL)	279.8 ±17.2	−73.5 ±17.8*	−16.7%*	236.6 ±16.9	+7.8 ±17.8	+13.4%
HDL-C (mg/dL)	39.06 ±1.1	+2.1 ±0.9	+6.6%	38.4 ±1.0	+1.7 ±0.9	+5.5%
Non-HDL-C (mg/dL)	151.35 ±3.4	−0.4 ±3.5*	−3.8%*	151.2 ±3.3	30.3 ±3.4	+12.6%
LDL-C (mg/dL)	102.6 ±2.4	+4.7 ±2.4*	+8.3%*	101.9 ±2.4	+15.4 ±2.4	+17.8%
Total-C (mg/dL)	191.3 ±3.6	+1.95 ±3.3*	+2.2%*	190.7 ±3.5	31.4 ±3.3	+17.4%
LDL particle conc. (nm ol/L)	1313.7 ±36.7	−25.2 ±36.9**	+3.0%**	1303.9 ±36.0	105.1 ±37.1	+11.0%
LDL – particle size (nm)	20.01 ±.08	+0.43 ±0.07	+2.2%	20.04 ±.08	+0.35 ±0.07	+1.8%
Apolipo-protein B (mg/dL)	1.02 ±.02	−0.02 ±0.02*	+1.4%*	1.01 ±.02	+0.12 ±0.02	+12.5%

*P≤0.005 or **P<0.05, between treatment groups

Conclusion: Treatment with either PIO or ROSI yielded comparable A1C reductions in this subgroup of patients

naïve to OAM in the GLAI study. After 16 weeks of thiazolidinedione treatment in OAM naïve patients, there was a marked reduction in A1C despite only moderately elevated baseline A1C levels. Approximately half of patients in both treatment groups achieved the ADA target of A1C<7.0% Similar to results from GLAI in both OAM treated and naïve patients, PIO demonstrated significant improvements versus ROSI in several lipid parameters (TG, TC, non-HDL-C, LDL-C, LDL particle concentration, and ApoB) in this OAM naïve subgroup.

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Lipid and apolipoprotein levels improve with pioglitazone plus sulfonylurea after treatment conversion from rosiglitazone in subjects with type 2 diabetes and dyslipidemia who continue stable statin therapy

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Background and Aims: A randomized, head-to-head, placebo-controlled study demonstrated that the thiazolidinediones pioglitazone (PIO) and rosiglitazone (ROSI) exerted differential lipid effects in patients with type 2 diabetes and dyslipidemia in the absence of lipid-altering therapy. The PIO-treated patients showed improvements over ROSI-treated patients in triglycerides (TGs), high-density lipoprotein cholesterol (HDL-C), and LDL particle concentration and size, and smaller increases in low-density lipoprotein cholesterol (LDL-C) and non-HDL-C levels (all results P≤0.005). The COMPLEMENT study was designed to determine whether the lipid differences observed in the previous study would be similar when subjects were converted from ROSI to PIO therapy while maintaining constant doses of their existing statins and other lipid-altering therapies. The primary outcome measure was fasting TGs; additionally, total cholesterol, LDL-C, HDL-C, apolipoprotein (apo) A-I, and apo B were measured. Here we evaluate the lipid-altering effects of PIO when added to an existing sulfonylurea regimen in a subset of patients from a post-hoc analysis of COMPLEMENT.
Materials and Methods: In this multicenter, open-label study, 305 patients with type 2 diabetes and dyslipidemia (TGs 2.26–11.29 mmol/L) who had been taking stable doses of ROSI and statin therapy for at least 90 days were converted to PIO at baseline. Of these patients, 130 were on a sulfonylurea-based regimen. All patients were then followed for 17 additional weeks while continuing a constant dose of the existing statin agent (+additional lipid-altering therapies, if already present). PIO was started at 30 mg/d, but could be increased to 45 mg/d at the investigator's discretion.

Results:

	Baseline Mean (SD) (n=130)	Week 17 Mean Change (SE) (n=121)	Week 17 Mean % Change (n=121)	P-value[a]
HbA1c (%)	7.5 (1.13)	0.05 (0.070)	NC	NC
TGs (mmol/L)	3.55 (1.877)	−0.71 (0.129)[†]	−14.17	<0.001
Total Cholesterol (mmol/L)	5.12 (1.059)	−0.56 (0.060)[†]	−10.00	<0.001
LDL-C (mmol/L)	2.65 (0.814)	−0.093 (0.049)	2.44	0.532
HDL-C* (mmol/L)	1.08 (0.241)	−0.013 (0.013)	0.53	0.678
Apo A-I (g/L)	1.175 (0.464)	0.091 (0.017)[†]	NC	NC
Apo B (g/L)	0.907 (0.247)	−0.038 (0.013)[††]	NC	NC

*Baseline N=129, Week 17 N=120
[†] P<0.001
[††] P=0.003
[a] P value for % change
NC=not calculated

At week 17 following conversion from ROSI to PIO, significant reductions were observed in TGs and total cholesterol: LDL-C decreased and HDL-C increased, but these changes were not statistically significant. Apo A-l levels increased significantly and Apo B levels decreased significantly. In addition, LDL-C particle concentration decreased and LDL-C particle size increased. A1c levels was virtually unchanged after conversion to PIO.
Conclusion: Replacing ROSI with PIO in subjects with type 2 diabetes while maintaining concomitant statin and other lipid-altering therapies resulted in significant improvements in TGs, total cholesterol, minor improvements in LDL-C but no change in HDL-C levels in this subset of patients on a sulfonylurea-based regimen. Both apo A-1 and apo B levels also improved significantly. These combined improvements were independent of glycemic control, which remained stable after treatment conversion.

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A one-year, randomized, placebo-controlled, double-blind trial of rosiglitazone in non alcoholic steatohepatitis: Results of the FLIRT pilot trial

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Background and Aims: Nonalcoholic steatohepatitis (NASH) is a liver disease associated with insulin resistance. Glitazones are promising drugs for NASH but only uncontrolled studies are available thus far. The aim was to test the efficacy and safety of rosiglitazone vs. placebo in patients with NASH.
Materials and Methods: Patients with biopsy-proven NASH were randomized to a one-year treatment with rosiglitazone (RSG, 8 mg/day) or placebo (PLB), followed by a control liver biopsy. End-points were, primary : improvement in steatosis > 30% (histological response, HR); secondary : normalization of transaminase ALT (biochemical response, BR).
Results: 32 patients were included in the RSG group and 31 in the PLB group. Sex ratio was 1.4, mean age 54 years. 20 Pts had diabetes (32%, 17 drug-treated, 16 with metformin), 35% arterial hypertension, mean BMI was 31.1 kg/m^2. RSG significantly improved serum glucose (−0.93 mmol/l vs+0.55 mmol/l in PLB, p=0.001) and, in non diabetics, median insulin (−5 μUI/l vs 0 μUI/l in PLB, p=0.016), HOMA (−1.18% vs 0.3% in PLB, p<0.03) and QUCKI (0.032 vs −0.005 in PLB, p<0.01). HR occurred in 47% (15/32) RSG and 16% (5/31) PLB pts (p<0.004). BR occurred in 38% (12/32) RSG and 7% (2/31) PLB pts (p=0.005). In RSG treated pts, HR was more frequent in non-diabetics, (61%, 14/23), than in diabetics (11%, 1/9, p<0.01) while BR was equivalent (39%, 9/23, vs 33%, 3/9, respectively). The antisteatogenic effect of RSG was correlated with improvement in ALT (r=0.35, p<0.05), QUICKI (r=0.66, p<0.01) and HOMA (r=−0.55, p=0.04). In the RSG group, 5 pts required dose reduction due to side effects (vs. 1 in the PLB group, p<0.09) and 1 had to discontinue treatment. Weight gain was+1.5 kg in RSG pts and −1.4 kg in the PLB pts (p3 kg was seen in 31% RSG (10/32) and 13% (4/31) PLB groups (p<0.08). Pedal edema developed in 10 RSG and 8 PLB pts. ALT elevation consistent with hepatotoxicity was not observed in any pt.
Conclusion: A one year treatment with RSG, an insulin sensitizing agent, significantly improves liver injury in pts with NASH. Large scale trials are warranted and longer treatment durations should be tested.
Supported by GSK

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Coronary artery disease and assessment of cardiac function

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Deterioration of adrenergic and cholinergic coronary artery tone and heart function regulation in diabetic dogs

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Background. It is well known that insulin deficiency and hyperinsulinemia in diabetes mellitus evoke neuropathy with disturbances of sympathetic and parasympathetic nervous and hormonal regulation of heart function and vessels tone. However peculiarities and exact mechanisms of heart function and vessels tone regulation disorders induced by diabetes mellitus remain insufficiently studied.
Aim. The experimental study was devoted to investigation of peculiarities of adrenergic (Adr) and cholinergic (Ch) coronary artery (CA) tone and heart function regulatory disorders in different stages of diabetes mellitus development.
Materials and Methods. Experiments were carried out on closed-chest dogs under alpha-chloralose anesthesia (50–100 mg/kg, i.v.), catheterization and programmed auto-perfusion of left CA, catheterization and continuous drainage of coronary sinus, heart and main vessels catheterization, registration of cardiohaemodynamics, CA resistance and ECG were performed. In metabolically healthy dogs (HD) and alloxane-diabetic dogs (AD, 75 mg/kg of alloxane, i.v.) Adr and Ch test-reactions of CA and heart were provoked by bolus intracoronary (i.c.) injection of epinephrine (E, 0.05–5.0 mcg), norepinephrine (NE, 0.05–5.0 mcg) and acetylcholine (Ach, 0.001–1.0 mcg). Due to different levels of hyperglycemia development in AD all of them were divided into three groups: HD - with normal level of glycemia (3.8± 0.5 mmol/l); AD1 - hyperglycemia − 7.2± 1.7 mmol/l; AD2 − 9.8+1.7 mmol/l and AD3−15.6±3.4 mmol/l.
Results. In HD E or NE injection into CA blood flow caused dose-dependent of short duration reflectory Ch CA dilation (1 phase) and attenuation of heart contractility then followed by more pronounced and protracted Adr CA resistance decrease and augmentation of heart contractility (2 phase). In AD1 Ch and Adr CA dilation induced by E or

NE injection (5.0 mcg, i.c.) increased (Ch:+47.1% and +58.3%; Adr:+66.7% and+23.7%, resp.). The same but more pronounced changes of Ch and Adr CA reactions in AD2 were observed (Ch:+62.5% and+100%; Adr:+74.4% and+105.2%, resp.). Both in AD1 and AD2 negative reflectory Ch heart reactions and positive Adr ino- and chronotropic heart reactions augmented. On the contrary, in AD3 Ch and Adr CA reactions were diminished (Ch and Adr, E: −29.2% and −33.3%; Ch and Adr, NE: −83.3% and −34.2%, resp.). Besides, negative and positive ino- and chronotropic heart reactions were limited or absolutely disappeared. In AD1 CA dilatations induced by Ach (1.0 mcg, i.c.) enhanced (+23.3%) whereas in AD2 and AD3 these reactions diminished (−4.7% and −25.6% resp.).

Conclusion. We suggest that identified peculiarities of Ch and Adr CA and heart function regulatory deteriorations participate in diabetic cardiovascular complications development and must be taken into considerations in practice.

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Validation of the French guidelines on the diabetic patients to screen for silent myocardial ischaemia

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Background and Aims: French Societies of Diabetology and Cardiology recommended in 2004 to screen for silent myocardial ischemia (SMI) the diabetic patients selected on the following criteria : those with nephropathy or carotid or peripheral artery disease, those with ≥ 2 other cardiovascular risk factors if type 1 diabetic patients older than 45 yrs or with diabetes duration > 10 yrs, and if type 2 diabetic patients older than 60 yrs or with diabetes duration > 15 yrs. The aim of this study was to evaluate the impact of these criteria on the prevalence of SMI, the prevalence of coronary stenoses (CS) on angiography and the incidence of cardiac events (CE: cardiac death, myocardial infarction, cardiac failure, coronary revascularization and unstable angina).

Patients and Methods: We retrospectively studied the files of 362 diabetic patients who were screened in 1997–2000 for SMI, defined as an abnormal myocardial scintigraphy, and for CS on angiography (performed in the patients with SMI). Patients were followed up for 41±24 months. In this population, 134 patients fulfilled the French screening criteria (C+) and 228 did not (C-).

Results: The prevalence of SMI was similar in both groups: 47/134 C+(35.1%) versus 74/228 C- (32.5%). The positive predictive value of SMI for identifying patients with CS was higher ($p<0.02$) in the C+group (24 out of 39 performed coronary angiographies, 62%) than in the C- group (20/55, 36%). SMI was predictive of CE (Kaplan Meier, log rank 9.1 ; $p<0.005$). The incidence of CE was greater in the C+group (11%) than in the C- group (4%) (Odds Ratio=2.8 [1.2–6.8], $p<0.02$). In the C+group, 128 were followed-up ; a CE occurred in 8 of the 45 SMI +patients, in 6 of them within 3 yrs, whereas a CE occurred in 6 of the 83 SMI- patients, always after 3 yrs.

Conclusions: The present data show that the current French guidelines do identify diabetic patients with high risk of silent CS and CE, and suggest that patients fulfilling these criteria and with a negative screening should be reassessed 3 years later.

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Selecting asymptomatic type 2 diabetic patients or screening for silent myocardial ischaemia: what risk factors should be considered?

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Background and Aims: Silent myocardial ischemia (SMI) is frequent in type 2 diabetic patients (T2DP) and could contribute to worse outcomes in this population. Recent studies suggest that traditional risk factors (RFs) are not associated with abnormal screening tests, leading the authors to suggest that all T2DP may merit screening stress tests. However, since stress tests in asymptomatic T2DP are mostly performed to identify patients with significant CAD susceptible to benefit from coronary revascularization, we evaluated whether traditional RFs are associated with asymptomatic but angiographycally proven CAD.

Materials and Methods: Seven hundred twenty one asymptomatic T2DP were evaluated for SMI using stress myocardial perfusion imaging; of 222 patients with positive results, 137 underwent coronary angiography, 71 of whom showed significant CAD. Angiography showed no significant lesion in 66 patients. Multivariate logistic regression was used to examine difference in RFs between the latter 2 groups.

Results: Nephropathy (odds ratio 4.0 [95% CI 1.8–9.5], P=0.001), HDL-cholesterol<50 mg/dl (3.6 [1.5–8.4], P=0.004), age >60 years (3.2 [1.3–7.6], P=0.009), treated dyslipidemia (2.8 [1.5–8.4], P=0.004) and HbA1c (2.9 [1.2–7.5], P=0.025) were significant independent predictors

of asymptomatic CAD in T2DP. A significant association was found between CAD and the number of these factors ($p<0.0001$). No association was found between CAD and the number of RFs when considering the RFs of current ADA screening guidelines.
Conclusion: Considering that the RFs we identified are all recognized predictors of cardiovascular events in T2DP, we conclude that screening stress tests should be limited to patients who have an association of these RFs, i.e. to patients who have a high risk of cardiovascular events and susceptible to benefit from coronary revascularization.

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Increasing prevalence of cardiovascular disease in Mauritius, and a strong association between postprandial hyperinsulinaemia and ECG changes indicating possible ischaemic heart disease

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Background: With increasing westernization, increasing prevalence of obesity is followed by coronary heart disease (CHD) and diabetes. Ethnic differences may be important as people with an Asian background seem to develop obesity-related diseases at lower levels of obesity.
Materials and Methods: Three population-based surveys were performed in Mauritius, in 1987, 1992 and in 1998. Altogether 9688 participated, and more than 50% were seen more than twice. Life-style related questionnaires, anthropometry, oral glucose tolerance tests, and 12-lead ECGs (>35 years) were included. Blood lipids, glucose, insulin and leptin were analysed. ECG changes were classified as "probable CHD" (mainly anterior Q-waves) and "possible CHD" (mainly STT depression/inversion and LBBB) according to Minnesota code.
Results: Probable changes were more common in men than women, and increased with age, whereas possible changes were more common in women, especially those with an African ancestry.
The incidence of probable ECG changes increased from 1.5 (1987–1992) to 4.4 (1992–1998) per 1000 PY and the incidence of possible ECG changes increased from 24.9 to 38.4 per 1000 PY.
In an cross-sectional analysis, approximately 60% of all subjects with probable ECG changes had abnormal glucose tolerance (impaired fasting glucose, impaired glucose tolerance or diabetes). High levels of postprandial glucose and insulin levels associated with the presence of both types of ECG changes in a multivariate analysis, taking into account traditional risk factors such as age, smoking, hypertension, diabetes, dyslipidemia and obesity. Fasting insulin and glucose levels did not associate with ECG changes once adjusted for traditional risk factors. In addition, only postload insulin levels related to self-reported heart attacks after adjustments.
Conclusion: Postprandial levels of glucose and insulin strongly associated with ECG changes indicating possible or probable CHD in a population of mainly Asian and African ancestry. Over 12 years, the incidence of CHD-related ECG changes appeared to increase in Mauritius.

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Hyperglycaemia and its effect after acute myocardial infarction on cardiovascular outcomes in patients with type 2 diabetes mellitus (HEART2D): Geographic differences in baseline CV risk factors and treatments

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Background and Aims: HEART2D study aims to determine whether an insulin regimen targeting postprandial (pp) hyperglycemia reduces incidence of cardiovascular (CV) outcomes in patients with type 2 diabetes mellitus (T2DM) and acute myocardial infarction (AMI) when compared to a regimen that targets fasting/pre-meal blood glucose (BG). Patients are followed up to 5 years after study enrollment. The current analysis evaluates regional differences in baseline CV risk factors (CVRF) and CV treatments.
Materials and Methods: Post AMI (within 21 days of event) T2DM patients were randomized to 1 of 2 treatments; (*a*) prandial strategy: pre-meal insulin lispro and NPH at bedtime, if needed or (*b*) basal strategy: NPH twice-daily or insulin glargine once-daily, followed by twice-daily human premixed insulin, if needed. Treatment targets: prandial strategy, A1C<7.0% and pp BG<7.5 mM; basal strategy, A1C<7.0% and fasting/premeal BG 4.4–6.7 mM. The 5 regions were Eastern Europe (n=718), Middle East (n=149), South Africa (n=89), Western

Europe/Canada (n=84) and Western Asia (n=70). Chi-square and ANOVA were used to test regional differences.
Results: One thousand one hundred twenty patients from 105 centres in 17 countries were randomised. The proportion of patients with ST-segment elevated entry MI was variable (60–83%; p=.01), as it was for left ventricular ejection fraction (49.5–54.3%; p=.0003) and QT interval duration (420–439 ms; p<0.0001). No differences were found for duration of diabetes, albumin/creatinine ratio or A1C, but fasting BG (8.1–8.8 mM; p=.01) and mean pp BG (10.6–13.1 mM; p<0.0001) differed significantly. Postprandial BG levels were highest in the subpopulation from Western Asia. The following differences in CVRF were found: triglycerides (mean range) 1.6–1.9 mM (p=.07), total cholesterol 3.7–4.7 mM (p<0.0001), low-density lipoprotein cholesterol 2.1–2.9 mM (p<0.0001), and tobacco use 7–20 yrs (p=.03). Treatments received for entry AMI also showed variability: primary percutaneous transluminal coronary angiopathy 31–57 (p=0.0005), fibrinolysis 9–49% (p<0.0001), CABG 1–10% (p<0.0001), and GP IIb/IIIa receptor blockers 12–32% (p<0.0001). Application of i.v. insulin infusion for AMI was similar across regions: 28–37% (p=0.4).
Conclusions: These regional differences in features of the entry AMI, daily BG profiles, CVRF, and treatment for AMI will be critical to fully understand the results of this trial, and may have implications for calculating sample size and design of future trials on CV disease and diabetes.
This study was funded by Eli Lilly and Company.

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Effects of intensive insulin therapy in non-previously known diabetic subjects with an acute myocardial infarction and hyperglycaemia. The INSUCOR study
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Background and Aim: Hyperglycaemia has been widely associated with a bad prognosis in acute coronary syndrome. The clinical benefit of maintaining glycaemia close to normal levels using insulin in the course of acute myocardial infarction (AMI) has been evaluated mainly in diabetic subjects. Our study aimed to investigate the short-term effects and the safety of strict glycaemic control using i.v.insulin in subjects with an AMI and hyperglycaemia without known diabetes. Patients and methods: 28 subjects with AMI and a plasma glycaemia (PG) at admission > 140 mg/dl and after 6 h-fasting > 126 mg/dl were included in the study. They were randomly assigned to two different treatment groups of 48 hours' duration: Intensive Group (IG, n=13), i.v. Intensive insulin therapy to keep PG between 80–110 mg/dl and Control group (CG, n=15), s. c. insulin only if PG > 160 mg/dl. Glucose profile and hs C reactive protein (CRP) were evaluated at admission and after 48 h of intervention. Clinical and metabolic parameters were analysed during hospitalisation and after 4 weeks.
Results: There were not initial differences in clinical characteristics and PG (Age, 62±12 yr vs. 67±10; Women, 3 vs. 5; PG, 180±69 mg/dl vs. 192±62, IG and CG, respectively). During the 48 hours of experimental treatments, PG was significantly lower in IG (104±8 mg/dl) than in CG (153±54 mg/dl, p=0.002) without hypoglycaemic episodes in any group. After 48 h, hsCRP was significantly lower in IG (20.4±20.0 mg/l) than in CG (44.3±28.0; p=0.04). This difference disappeared at the moment of discharge. After 4 weeks, glucose tolerance categories (OGTT) were similar in both groups, with only 28.6% of the subjects being normal in OGTT. HbA_{1c} at admission was significantly higher in those subjects later diagnosed of diabetes.
In summary, the maintaining of near normoglycaemia is feasible and safe using i.v. insulin in subjects without known diabetes with AMI and hyperglycaemia. In addition, it produces attenuation in inflammatory response. Our study also confirms the high prevalence of unknown abnormalities in glucose tolerance in subjects with AMI.
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Predictors of transition to persistent fibrillation in type 2 diabetic patients with paroxysmal atrial fibrillation
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Background and Aims: Atrial fibrillation (AF) is often observed in patients (pts) with type 2 diabetes mellitus (T2DM). It has been shown, that P-wave dispersion (Pd) may be used for predicting pts with paroxysmal AF (PAF) from healthy subjects. This study was conducted for identifying PAF predictors of progression to persistent AF in T2DM

Materials and Methods: Participants were 64 pts (37 males/27 females, mean age 59.7+6.3 yrs) with T2DM and PAF in history. In all pts 12-lead surface ECG, 24-hour ECG and blood pressure (BP) monitoring and echocardiography were performed. The maximum P-wave duration (Pmax), minimum P-ware duration (Pmin) and P-wave dispersion (Pd=Pmax-Pmin) were calculated on the surface ECG. The rhythm disturbances were studied using Holter ECG. Left ventricular ejection fraction (LVEF), left ventricular mass (LVM) and left atrial diameter were measured by echocardiography. The pts were followed for 12 months.
Results: At the end of the study AF became chronic in 34.7% of pts (n=22, Gr.1). At entry, the pts who developed chronic AF in the future, differed from those who did not (n=42; Gr. 2). Pts age in Gr. 1 was higher than in Gr. 2 (63.3+5.1 vs 54.4+6.3 yrs, P=0.000, respectively). Hypertension was more frequent, and the number of non-dippers was higher in Gr. 1, than in Gr. 2. In Gr. 1–12 (54.5%) out of 22 pts, and in Gr.2–11(26.2%) out of 42 pts were non-dippers. 24 -hour systolic BP (SBP) was higher in Gr.1 (143+9 vs 138+6 mmHg); according to Holter ECG data no statistically significant difference in frequency of supraventricular extrasystols between the groups was registered, though episodes of silent myocardial ischemia were more frequent in Gr.1 ($p<0.01$). LVM and left atrial diameter were higher in Gr.1, than in Gr.2 ($P<0.01$, $P<0.05$, respectively). LVEF was lower in pts with chronic AF in the future, but not significantly. There was significantly increased P-wave dispersion (59+7 vs 48+8 ms, P=0.000, respectively). Persistent AF in T2DM pts is associated with age, left ventricular mass, lack of nocturnal decline of SBP, silent ischemia episodes, left atrial size, and increased P-wave dispersion.
Conclusion: In T2DM pts risk of chronic atrial fibrillation increases with age, left ventricular mass and left atrial size. Increased P-wave dispersion in T2DM pts with PAF in history is associated with increased risk of PAF transition to persistent atrial fibrillation.

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Lower heart rate variability is associated with higher plasma concentration of Il-6 in type 1 diabetis

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Background and Aims: In type 1 diabetes, cardiovascular autonomic neuropathy (CAN) is associated with cardiovascular risk factors related to insulin resistance, which in turn are associated with low-grade systemic inflammation. As the autonomic nervous system interacts with systemic inflammation, we evaluated CAN to test its possible association with low-grade systemic inflammation.
Materials and Methods: One hundred and twenty subjects diagnosed with type 1 diabetes 14 years before (age 27.4+6.6, 50% women), were analysed for: 1) clinical characteristics; 2) plasma levels of soluble fractions of TNF-α receptors 1 and 2, IL-6 and high-sensitivity C-reactive protein; 3) insulin resistance (through an estimation of the glucose disposal rate -eGDR-), and 4) tests for CAN (heart rate variability -HRV- in response to deep breathing (E/I ratio), HRV in response to the Valsalva manoeuvre and changes in systolic blood pressure responding to standing).
Results: A significant negative correlation was found between E/I ratio and plasma concentrations of Il-6 ($r=-0.244$, $p=0.013$). This correlation remained significant after adjusting for potential confounders (age, sex, smoking, alcohol intake, HbA1c, waist-to-hip ratio, diastolic blood pressure, triglycerides, HDL-colesterol, retinopathy, nephropathy, peripheral neuropathy and insulin dose ($r=-0.239$, $p=0.031$). No other significant associations were found between inflammation-related proteins, test for CAN and eGDR.
Conclusion: The current findings suggest a link between low grade inflammation and early alterations of CAN in type 1 diabetes amd may be of importance in the pathogenesis of CAN and/or its clinical implications.
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Usefulness of pulmonary venous flow in non-invasive assessment of early left ventricular diastolic dysfunction in young non-smokers with type 1 diabetis mellitus

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Background and Aims: The impairment of left ventricular diastolic function (LVDF) connected with diabetic cardiomyopathy leads to symptomatic heart failure. We studied mitral valve flow (MVF) and pulmonary venous flow (PVF) parameters in young not smoking patients with diabetes mellitus type 1 (DM1) to evaluate LVDF, using an echocardiographic method.

Materials and Methods: The study group (I) consisted of 72 patients (30 M, 42F, mean age 28.7±7.0 yrs) with DM1, non-smokers, without cardiac disease, with mean DM1 duration 15.4±7.2 yrs. Mean HbA1c was 9.6±2.6%. Thirty healthy non-smokers (15 M, 15F, mean age 29.1±6.7 yrs) served as a control group (II). In MVF: the maximal velocity (Vmax) of early phase - E, Vmax of late phase - A, E/A ratio, deceleration time (DT) and isovolumetric relaxation time (IVRT) were measured. In PVF: Vmax of systolic flow (S), Vmax of diastolic flow (D) and S/D ratio were evaluated.
Results: Mean values of BMI, heart rate, systolic and diastolic blood pressure, ejection fraction and LV mass did not differ between both groups. In MVF, the mean value of A was significantly higher (58.9±9.5 vs 53.5±10.4 cm/s; p=0,02), and the E/A ratio was significantly lower (1.35± 0.3 vs 1.54±0.35; p=0,01) in group I than in group II. Mean value of DT was significantly higher in group I than in II (168.4±17.7 vs 154.7±15.9 ms; p<0,001). The MVF results tend to show the impairment of LVDF in young not smoking DM1 patients, despite the fact they remain normal for this age group. In PVF, the mean value of D was significantly lower (47.5±7.6 vs 55.1±7.3 cm/s; p<0,0001), so the S/D ratio was significantly higher (1.13±0.2 vs 0.9± 0.1; p<0,0001) in diabetics than in controls. PVF S/D ratio showed systolic dominance (S/D>1) typical for impaired LV relaxation and was abnormal for this age group. No correlation between echo-parameters and DM1 duration, HbA1c level, presence of retinopathy, nephropathy or peripheral neuropathy was found.
Conclusion: 1. Echocardiographic assessment of PVF shows an impaired LV relaxation in young, not smoking patients with long term DM1. 2. PVF parameters are especially sensitive markers in detection of early subclinical LV diastolic dysfunction, especially when MVF parameters remain normal.

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Predictors of morbidity and mortality

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Abnormal glucose tolerance prior to first myocardial infarction - not as prevalent as expected

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Background and Aims: Abnormal glucose tolerance and especially 2 h-glucose is reported to predispose for cardiovascular disease (CVD). In a recently published study from Sweden it was reported that among patients, aged 63.5 (±9.4) years, who were admitted to hospital for acute myocardial infarction (AMI), a majority (66%) had an abnormal glucose tolerance i.e. previously unknown diabetes or impaired glucose tolerance (IGT). However, the prevalence of abnormal glucose tolerance in the general population is much lower, so, it has been speculated that AMI as a stressful life event by itself might inflict abnormal glucose tolerance. Our aim was to study the proportion of abnormal glucose tolerance prior to the subjects first AMI.
Materials and Methods: A prospective nested case-control study of 508 (M/F 414/94) patients with incident first-ever myocardial infarction (mean age 58.0±7.7 years), and 1751 sex and age matched controls (M/F 1434/317) was performed. Cases and controls had no diagnosed diabetes before participating in a health survey, which included an oral glucose tolerance test (OGTT) prior to the AMI (mean 4.1±2.7 years). Categorisation of the OGTT was based on the 1999 WHO criteria for diabetes and impaired glucose tolerance. It was thoroughly investigated that none of the cases or controls had AMI prior to the observed event. Myocardial infarction, fatal and non fatal, was diagnosed according to the protocol of the Northern Sweden MONICA data registry.
Results: 5.3% (95%CI 3.4–7.2; M/F 5.6%/4.3%) of cases and 3.0% (95%CI 2.2–3.8; M/F 3.0%/3.2%) of controls were found to have a previously unknown diabetes (p<0.05) whereas 9.4% (95%CI 6.9–11.9; M/F 8.0%/ 16.0%) of cases and 5.6% (95%CI 4.5–6.7; M/F 5.1%/ 7.9%) of the controls had impaired glucose tolerance (p<0.01). There was no significant difference in OGTT beween men and women. Hypertension (43% vs. 24%; p<0.001) and obesity (22% vs. 10%; p<0.001) was more frequent among subjects with diabetes/IGT compared to controls. Hypercholesterolemia (87% vs. 84%) and smoking (21% vs 26%) did not differ significantly.
Conclusion: In this population of AMI patients without previous cardiac events, abnormal glucose tolerance was almost twice as common as in age and sex matched controls. As expected, the subjects with abnormal glucose tolerance also had a higher risk profile with more signs of the metabolic syndrome. However, the proportions of abnormal glucose tolerance in both cases and controls in our study were about one quarter of the earlier published data from Norhammar et al. Our patients were younger and did not include patients with re-infarction which might contribute to the lower proportion of abnormal glucose tolerance in our study. In summary, the noticeable difference in glucometabolic state in AMI patients between our and previous studies calls for further evaluation of the impact of coronary events on patients' metabolic state.
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Abnormal glucose regulation is common and often undiagnosed in patients with coronary artery disease in China

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Background and Aims: Coronary artery disease (CAD) is a common cause of death in type 2 diabetes patients, and there is evidence that prediabetes, particularly impaired glucose tolerance (IGT), is also associated with an increased risk of cardiovascular morbidity and mortality. The presence of diabetes or prediabetes at admission for a cardiovascular event has been shown to be associated with poor patient outcome. Studies determining the glucometabolic state of patients with CAD in Europe have prompted this similar investigation in China.
Materials and Methods: The study was conducted in 52 centres in 7 cities in China, and enrolled 3513 patients hospitalised for CAD. Data were collected by case record forms. Glucometabolic state was classified using WHO 1999 criteria; prediabetes includes IGT and impaired fasting glucose. Fasting plasma glucose (FPG) levels were assessed on admission. Standard oral glucose tolerance tests (OGTTs) were performed in patients without known or newly diagnosed (FPG >7.0 mmol/L) type 2 diabetes, before discharge or within 2 months of admission.
Results: The majority of patients were male (67%). The mean age was 69 years and the mean body mass index was 24 kg/m^2. Of the patients enrolled, 35% were admissions for acute CAD and 65% were elective admissions for CAD. Acute coronary syndrome was the most common type of CAD diagnosis in both admission groups. At admission, 1153 (33%) patients had known type 2 diabetes and 97 (3%) had newly diagnosed diabetes by FPG level. Of the remaining patients, forming the OGTT cohort (n=2263), 27% had type 2 diabetes and 37% had prediabetes. The prevalences of type 2 diabetes and prediabetes were similar in the acute (28% and 39%, respectively) and elective (26% and 36%, respectively) admissions groups within the OGTT cohort. When FPG levels (OGTT 0 min) were considered, 10% of patients had diabetes or prediabetes. In contrast, when postchallenge levels (OGTT 2 h) were considered, 64% of patients had diabetes or prediabetes. Consequently, 87% of patients with prediabetes and 81% of patients with diabetes would have remained undiagnosed without the performance of OGTTs. Overall, the proportion of patients with diagnosed diabetes increased from 33% at the start of the study to 53% post-OGTT analysis. Glucometabolic state was not associated with the type of CAD diagnosis, but was significantly associated with HbA1c level, systolic blood pressure, triglyceride level, HDL-cholesterol level and serum creatinine level (all $p \leq 0.001$).
Conclusion: This study adds to the growing evidence that type 2 diabetes and prediabetes are common and often undiagnosed in patients with CAD. The prevalence of abnormal glucose regulation is underestimated by FPG testing alone, and OGTTs should be routinely used to assess the glucometabolic state of patients admitted with CAD. Earlier diagnosis and treatment of diabetes or prediabetes may improve long-term outcomes in this high-risk patient group.
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Diabetes, coronary heart disease and C-reactive protein in high-risk individuals undergoing coronary angiography

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Background and Aims: As a non-specific acute phase reactant, C-reactive protein (CRP) is being discussed as an adjunct predictor for cardiovascular disease which is an inflammatory process. Diabetes (DM) is a condition associated with an increased risk for cardiovascular morbidity and mortality and according to NCEP/ATP 3 guidelines considered a coronary artery disease equivalent. The aim of this study was to evaluate the impact of diabetes on the association of CRP with the presence of coronary heart disease (CHD) in a high-risk population undergoing coronary angiography.
Materials and Methods: One hundred and twenty three consecutive patients (female n=50, 40.7%, male n=73, 59.3%, mean age 59.40±10.91 years) referred to the department of cardiology for diagnostic coronary angiography were enrolled in this cross-sectional, case-control study upon verbal informed consent. Except for sociodemographics like age and gender, tobacco and alcohol consumption, family history of premature CHD, established diagnosis of diabetes mellitus, hypertension, hyperlipidemia and ongoing drug treatments were assessed. Other conditions, likely to confound CRP levels were exclusion criteria. Coronary angiography results were evaluated by two independent cardiologists and patients were dichotomized as cases (stenosis≥50% of one ore more main coronary artery) and controls (no stenoses). Main outcome

parameter was serum CRP level. Except for descriptives, Independent-Samples T Test was used for parametric and Chi-Square Test for non-parametric variables. Odds ratios (OR) were calculated via cross-tabulation with a 95% confidence interval (CI) and a significance level of 0.05. In multiple linear regression analysis, interaction models were built with CRP as dependent and CHD and DM as independent variables.

Results: CHD was angiographically detected in 93 cases (75.6%). Cases were differing from controls with respect to CRP (0.74±0.65 vs. 0.41±0.25, $p<0.001$) and HDL levels (57.16±16.72 vs. 49.63±13.22, $p=0.013$) in parametric testing. Older age (females≥55, males≥45 years) (OR=5.3, 95%CI 1.8 to 15.8), male gender (OR=4.0, 95% 1.9 to 8.3), smoking (OR=1.2, 95% CI 1.1 to 1.5) and hypercholesterolemia (OR=3.1, 95%CI 1.3 to 7.7) were associated with CHD in nonparametric testing. Nevertheless, cases were more likely to receive statin therapy (OR=1.2, 95%CI 1.0 to 1.5). Conventional risk factors like family history of premature CVD, diabetes and hypertension were not found to be associated with CHD. CRP among cases was 0.27 mg/dL (95%CI 0.02 to 0.52, $p=0.034$) higher than in controls after adjustment for age, gender and hypercholesterolemia in multiple regression analysis. DM and CHD significantly interacted in their relationship with CRP (P-interaction=0.078), as shown by a significant positive association of CHD with CRP in non-diabetic individuals (CRP 0.35 mg/dL higher in CHD(+) patients, 95%CI 0.1 to 0.6; $p=0.012$) which eroded in the presence of diabetes ($p=0.351$).

Conclusion: The interpretation of CRP as a screening test for coronary heart disease in diabetic individuals has to be critically reevaluated and investigated further.

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Type 2 diabetes and the coronary angiographic state are mutually independent predictors of future vascular events

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Background and Aims: Type 2 diabetes (T2DM) in cross-sectional studies is associated with coronary artery disease (CAD) and prospectively confers a strongly increased risk of vascular events. The aim of this study was to investigate to what extent the baseline CAD state accounts for the increased vascular risk of diabetic patients.

Materials and Methods: We enrolled 750 consecutive patients undergoing coronary angiography for the evaluation of CAD. At angiography, CAD was diagnosed in the presence of any irregularities of the vessel wall. Stenoses of at least 50% were considered significant, and the extent of CAD was defined as the number of significant stenoses in a patient. Vascular events were recorded over 4 years.

Results: The prevalence of CAD (87.8% vs. 80.4%; p=0.029) and of significant stenoses (69.5% vs. 58.4%; p=0.010) as well as the extent of CAD (1.7±1.5 vs. 1.4±1.5; p=0.014) were significantly higher in patients with T2DM (n=164) than in nondiabetic subjects (n=586). Prospectively, T2DM after multivariate adjustment strongly predicted vascular events (adjusted hazard ratio (HR)=1.66 [1.15–2.39]; p=0.006). Also, the presence of CAD (HR=2.78 [1.39–5.57]; p=0.004), the presence of significant stenoses (HR=3.67 [2.30–5.85]; p<0.001) and the extent of CAD (standardized adjusted HR=1.62 [1.41–1.87]; p<0.001) significantly predicted vascular events. These angiographic characteristics still predicted vascular events after additional adjustment for T2DM (HR=2.66 [1.33–5.34]; p=0.006, 3.57 [2.24–5.70]; p<0.001, and 1.60 [1.34–1.84]; p<0.001, respectively). Conversely, T2DM remained strongly and significantly predictive of future vascular events after adjustment for the presence and extent of CAD (HR=1.50 [1.04–2.15]; p=0.029).

Conclusion: Among angiographied coronary patients, the presence and the extent of CAD are higher in patients with T2DM than in nondiabetic individuals. Prospectively, T2DM and the baseline CAD state are mutually independent predictors of future vascular events.

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Mortality of patients with early-onset type 2 diabetes in Japan

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Background and Aims: According to a recent study based on the results of school health checkups involving urinalysis in urban areas in Japan, the number of patients with early-onset type 2 diabetes (E-T2D) has gradually increased, as reported for the number of patients with adult-onset T2D. In a survey in which diabetic complications were matched to the duration of disease, we previously reported that nephropathy in those with E-T2D was more advanced than in those with early-onset type 1 diabetes (E-

T1D). The above results suggest that the prognosis of E-T2D is poorer than that of E-T1D, in whom the standardized mortality ratio (SMR) was already reported to be 2.8. The aim of this study is to investigate the mortality and the causes of death in patients with E-T2D.

Patients and Methods: Among 927 patients with E-T2D who consulted our center between January 1, 1980 and December 31, 1990 (age at detection: less than 30 years), the subjects were 642 patients who were treated at our center for 1 year or more. The survival status by January 1, 2001 was investigated. The end-point was determined using questionnaires mailed to attending physicians or through telephone interviews with patients or their families.

Results: There were no differences of baseline characteristics between the subjects(n=642, 358 males and 284 females, age at detection and duration at start of follow-up in the center ; 0–9/10–19/20–29 ages=2/201/439 and 19±8.8/6.7±8.4/7.2±8.2, respectively) and the patients treated less than 1 year(n=285, 173 males and 112 females, age at detection and duration at start of follow-up in the center ; 0–9/10–19/20–29 ages=0/77/208 and 0/6.8±8.6/7.4±8.8, respectively). The completeness of this study was 84.4% (542/642). Fifty-one patients had died. The mortality ratio (/1,000 person-year) was 6.7 and the standardized mortality ratio (SMR) in the subjects was 3.7(95%CI; 2.2–4.5). The cumulative 10-year and 20-year survival rates from the follow-up period after the initial consultation in our center were 95.7% and 79.5%, respectively. Approximately 33% of the deceased patients died of major vascular disorders (CVD 17.6%; IHD 15.7%), although 67.5% of the patients had the end-stage renal disease and received dialysis treatment before dying.

Conclusion: The follow-up rate was lower than that in a survey involving patients with E-T1D in our center, though, the frequency of major vascular disorders as the cause of death was more frequent than that in the survey of E-T1D. This was possibly because the age at detection and that during follow-up in the subjects were older than those in the E-T1D in our center and dialysis treatment had effective for surviving but did not overcome the major vascular disorders.

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Elevated pregnancy-associated plasma protein A predicts mortality in type 1 diabetic nephropathy

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Background and Aims: Pregnancy-Associated Plasma Protein A (PAPP-A) has been implicated in acute coronary syndromes and carotid and peripheral atherosclerosis. Diabetic nephropathy is characterised by increased cardiovascular morbidity and mortality. This study investigated the prognostic value of circulating PAPP-A levels in a large cohort of type 1 diabetic patients with and without diabetic nephropathy.

Materials and Methods: In a prospective observational follow-up study 198 type 1 diabetic patients with overt diabetic nephropathy (122 men, age (mean(SD)) 41±10 years, duration of diabetes 28±8 years, GFR 74±33 ml/min) and a matched control group of 180 patients with longstanding type 1 diabetes and persistent normoalbuminuria (114 men, age 43±10 years, duration of diabetes 27±9 years) were followed for 9.3 (0.0–9.5) years. Plasma PAPP-A concentration was determined by immunoassay at baseline.

Results: In patients with diabetic nephropathy, plasma PAPP-A concentration was elevated 3.6 (0.4–51.1) mIU/l (median(range)) versus 2.1 (0.4–46.6) mIU/l in normoalbuminuric patients, $p<0.0001$. Furthermore, proMBP (proform of eosinophil major basic protein) the endogenous inhibitor of PAPP-A was raised in patients with nephropathy versus normoalbuminuria, 2287 (909–9287) and 1612 (780–12970) mIU/l, $p<0.0001$. For acute coronary syndromes, a PAPP-A threshold of 10 mIU/l has previously been suggested. Among patients with nephropathy 9 (47%) patients with plasma PAPP-A above the threshold and 42 (24%) with values below died from any cause (hazard ratio 2.41 (95%CI 1.17–4.95) p=0.017). This lower mortality was attributable to fewer cardiovascular deaths: 8 (42%) and 30 (19%) above and below the PAPP-A threshold level, respectively (hazard ratio 3.00 (1.37–6.52), p=0.006). PAPP-A levels at baseline were elevated in men as compared to women (4.0 (0.4–29.7) mIU/l versus 3.3 (0.5–51.1) mIU/l p=0.02, increased with age and systolic blood pressure, and rose with declining kidney function, $p<0.01$. The prognostic information of PAPP-A was attenuated after adjustment for confounding factors.

Conclusion: Elevated circulating PAPP-A predicts excess overall and cardiovascular mortality in type 1 diabetic patients with diabetic nephropathy.

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Homocysteine levels seem to be the main factor determining familial longevity

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Introduction: The increase of mean lifetime has also given rise to an increase in the number of centenarians. Longevity is the result of health-promoting behaviours plus genetic factors that promote disease resistance and long-term survival. If environmental factors did not change from one generation to the next, parent's and children's longevities would be expected to be similar. Some studies do, in fact, show similarities in the age of death in parents and offspring while other investigations confirm that parental survival is an independent predictor of offspring's longevity. Epidemiological studies have enhanced the importance of high-density lipoprotein (HDL) in coronary artery disease (CAD) and longevity. On the other hand, emerging risk factors such as homocysteine (Hcy) have been studied in relation to CAD but not to longevity.
Aims: 1- To evaluate metabolic, haemodynamic and endothelial parameters in a population of high cardiovascular risk which has been divided into different groups based on its family history of longevity. 2- To establish gender-related behavioural differences.
Material and Methods: N=227 patients of high cardiovascular risk (55% type 2 diabetic), aged 22–95 years (57,57±0,94). 113/114 male/female ratio. Family history of longevity (FHL): at least one first or second degree relative with a demonstrated survival over 85 years. Anthropometric parameters: waist circumference (centimetres) and waist/hip ratio.
Biochemical parameters: glucose, HDL, LDL, uric acid (Hitachi autoanalyzer). Hcy (uM/L): IMX Abott.PAI-1 (ng/ml): Biopool-Menarini ELISA kit. Ambulatory Blood Pressure Monitoring (ABPM): the following parameters were evaluated in 24h, during activity (9:00 to 22:00), rest (22:00 to 6:00) and critical (06:00 to 9:00) periods: average systolic blood pressure (aSBP), average diastolic blood pressure (aDBP), average mean arterial pressure (MAP) [(SBP-DBP)/3+DBP]. Ambulatory pulse pressure (APP). Standard systolic and diastolic blood pressure burden (systolic>125 and diastolic>75 mmHg pressure reading percentages) (SBPB and DBPB). Hemodynamic load (HL=Heart Rate x MAP/100). SBP variability (SBPV) and DBP variability (DBPV). HR variability (HRV). Statistical analysis: Student's T test. Mann-Whitney's U test. Kolmogorov-Smirnov's test. Chi-squared test. RESULTS: 1 Patients with a family history of longevity (FHL) (n=82, 37/45 male/female ratio) presented lower levels of Hcy (12,25±0,39 vs 13,87±0,83; p<0,05) compared to those without FHL (n=145, 76/69 male/female ratio). Ages were similar in both groups (57,97±1,22 vs 56,87±1,46). 2 In men, statistically significant differences were found in waist circumference values (91,62±1,49 vs 96,75±2,16, p=0,034) and Hcy levels (12,03±0,58 vs 15,61±1,32, p=0,007) between both groups. Differences were also found in PAI-1 levels (45,11±2,74 vs 56,88±4,73), although at the borderline of statistical significance (p=0,06). No significant differences were found in women.
Conclusions: 1. In the general population, homocysteine levels seem to be the main factor determining familial longevity. 2. In the gender-based study, this discovery is confirmed only in males. 3. Such differences in males were further accompanied by differences in waist circumference and endothelial impact, at the borderline of statistical significance.

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N-terminal pro brain natriuretic peptide levels in correlation with renal echo Doppler parameters and microalbuminuria in hypertensive diabetic and nondiabetic patients

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Background and Aims: Elevated N terminal pro brain natriuretic peptide (NT-proBNP) is related to the presence of cardial dysfunction, but is also a predictor of increased cardiovascular morbidity in microalbuminuric type 2 diabetic patients. Our aim was to study NT-proBNP in hypertensive diabetic or nondiabetic patients, without symptoms of cardial disease, in relation with microalbuminuria, and renal echo-Doppler measurements.
Materials and Methods: Waist circumference, blood pressure, ProBNP, microalbuminuria, creatinine measurements, renal echo Doppler (pulsatility index (PI), resistive index (RI)) was assessed and full echocardiographic examination was performed in 62 hypertensive patients (aged 52,5±3 years, 38 male). 34 (55%) of the patients had type 2 diabetes.
Results: Diastolic dysfunction was more frequent in diabetic patients (89% versus 68%, p<0,05). Pulsatility index and resistive index were higher in patients with type 2 diabetes (1,11±0,24 respective 0,70±0,1 versus 1,0±0,12 respective 0,65±0,07, p<0,05). ProBNP correlated also with age (r=0,561, p<0,001) and creatinine clearance (r=0,412, p<0,05). After adjusting for age and creatinine clearance, ProBNP was found significantly higher in diabetic patients (177,1±87, versus 98,3±36, p<0,01), but was not significantly correlating with echocardiographic parameters. We could not find any correlation between proBNP and microalbuminuria in our patients, but proBNP was highly

correlating with renal pulsatility and resistive index (r=0,423, and 0,452, p<0,01).
Conclusion:The present study provides evidence for the correlation of NT-proBNP with the presence of diabetes, and with intrarenal flow deterioration in hypertensive, type 2 diabetic patients, independent of other cardiovascular parameters.

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Glomerular filtration rate, cardiovascular endpoints and all-cause mortality in type 2 diabetic patients

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Background and Aims: Chronic kidney disease (CKD) predicts cardiovascular disease (CVD) in general population. However, its impact on diabetic populations, especially Chinese, has not been fully studied. We investigated the effects of stages of renal function using estimated glomerular filtrate rate (eGFR) on all-cause mortality and cardiovascular endpoints in a large prospective cohort of Chinese type 2 diabetic patients.
Materials and Methods: Between 1995 and 2000, 4,421 Chinese type 2 diabetic patients without macrovascular disease or end stage renal disease was enrolled. Renal function was assessed by eGFR calculated by the abbreviated Modification of Diet in Renal Disease Study Group (MDRD) formula. Clinical endpoints included all-cause mortality and cardiovascular endpoint (cardiovascular death, new admissions due to angina, myocardial infarction, stroke, revascularization or heart failure).
Results: After a median follow-up of 39.4 months [interquartile range: 20.3– 55 months], rates of cardiovascular endpoints and all-cause mortality increased from 2.6% to 24.6% (95% CI [2.0–3.3] to [14.2–35.1], p for trend<0.001), and 1.3% to 17.4% ([0.8–1.8] to [8.2–26.6], p for trend<0.001) amongst patients with eGFR ≥90 ml/min/1.73 m^2 to those with eGFR between 15 and 29 ml/min/1.73 m^2 respectively. After adjustment for covariates (age, sex, albuminuria, lipid, blood pressure and glycemic control), hazard ratios across different stages of eGFR (≥90, 60–89, 30–59, 15–29 ml/min/1.73 m^2) for all-cause mortality were 1.00, 1.27, 2.34 and 9.82 (p for trend<0.001), while for cardiovascular endpoints were 1.00, 1.04, 1.05 and 3.23 (p for trend<0.001) respectively.
Conclusion: Chinese type 2 diabetic patients with reduced eGFR were at high risk of developing cardiovascular endpoints and all-cause mortality, independent of albuminuria and metabolic control.

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Reduction of plasma N-terminal pro-brain natriuretic peptide by angiotensin II receptor blockade in type 2 diabetic patients with microalbuminuria

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Background and Aims: Raised plasma N-terminal pro-brain natriuretic peptide (NT-proBNP) is independently associated with an increased overall and cardiovascular mortality in non-diabetic and diabetic populations. We investigated the effects of the angiotensin II receptor blocker irbesartan given in the maximally recommended dose (300 mg daily) and in ultrahigh doses (600 and 900 mg daily) on NT-proBNP in type 2 diabetic patients with microalbuminuria.
Materials and Methods: This double-masked randomized cross-over trial included 52 (41 males) hypertensive Type 2 diabetic patients with microalbuminuria on ongoing antihypertensive medication. At inclusion, previous antihypertensive treatment was discontinued and replaced with bendroflumethiazide 5 mg o.d. for the entire study. Following two months wash-out (baseline), patients were treated randomly with irbesartan 300, 600 and 900 mg o.d., each dose for two months. End-points evaluated at the end of each study period included plasma NT-proBNP (immunoassay, Roche Diagnostics), and as previously reported 24-hours urinary albumin excretion (UAE), 24-hrs ambulatory blood pressure ((ABP)), Takeda-TM2420).
Results: Two months after withdrawal of previous antihypertensive medication, baseline values were: plasma NT-proBNP[geometric mean (95%CI)] 32 (23 to 44) ng/ml, UAE 93 (72 to 121) μg/min, and ABP [mean (SD)] 140 (10)/77 (7) mm Hg.
All three doses of irbesartan significantly reduced plasma NT-proBNP, UAE, systolic - and diastolic ABP as compared with baseline.
Reductions in plasma NT-proBNP from baseline were: 20 (4 to 32), 25 (3 to 43) and 30 (10 to 44)% with increasing doses of irbesartan (NS between doses). UAE were reduced by 52 (46 to 57), 49 (43 to 54) and 59 (54 to 63)% with increasing doses of irbesartan (p<0.01). UAE was reduced significantly more by irbesartan 900 mg compared with lower doses with an additional reduction in UAE of 15 (2 to 26)% by irbesartan 900 mg compared with 300 mg (p=0.02). With increasing doses systolic-ABP was reduced from baseline by 8 (4 to12), 9(5 to13) and 9 (5 to13)

mmHg, and diastolic-ABP by 6 (4 to7), 7 (6 to 9), and 7 (6 to 9) mm Hg (NS between doses).
Conclusion: Plasma NT-proBNP is reduced by irbesartan in type 2 diabetic patients with microalbuminuria. Furthermore there is a tendency towards an increased and dose-dependent reduction by ultra-high. doses of irbesartan.
The study was supported by an unrestricted study grant from Sanofi-Aventis, who also provided the study medication. The assay for measurement of NT-proBNP was provided by Roche Diagnostics GmbH, Mannheim, Germany

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Diabetic cardiomyopathy

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Critical role of calcium-dependent molecular pathways in type 2 diabetes-associated enhanced activity of the cardiac Na+/H+exchanger
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Background and Aims: The Na^+-H^+ exchanger (NHE) is a potential target in terms of attenuation of hypertrophic processes that contribute to heart failure. We investigated sarcolemmal NHE activity and its putative role on left ventricular (LV) abnormalities in a type 2 diabetic animal model.
Materials and Methods: Fluorometric measurements in isolated adult cardiomyocytes demonstrated that LV cardiac hypertrophy in GK (Goto-Kakizaki) rat hearts was associated with enhanced sarcolemmal NHE activity and increased cytosolic Ca^{2+} levels. Chronic treatment of GK rats with the NHE1 inhibitor cariporide normalized the increase in NHE activity and also significantly reduced LV myocyte hypertrophy. To characterize the molecular pathways driving enhanced NHE activity, we evaluated the role of mitogen-activated protein kinases (MAPKs), protein kinase C (PKC) isotypes, and Ca^{2+}/calmodulin-dependent kinase II (CaMKII).
Results: The results demonstrate that the activity of Erk, but not p38 MAPK, was increased markedly, and that Ca^{2+}-dependent PKC and CaMKII are involved. Thus NHE activity, as assessed by the rate of acid efflux carried by the exchanger at an intracellular of 6.85, was significantly more reduced in GK LV myocytes than in control LV myocytes when each of these kinases was specifically blocked. Only intracellular Ca^{2+} increase, higher sensitivity of NHE activity to CaMKII as well as to Ca^{2+}-dependent PKC regressed during reversal of the hypertrophic phenotype by cariporide treatment.
Conclusion: Preventing excessive activation of NHE and subsequent increases in intracellular Ca^{2+} as well as critical Ca^{2+}-dependent kinases activity may afford protection against LV hypertrophy in type 2 diabetes.
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Moderate hyperglycaemia affects early MMP activity without effects on late LV remodeling after myocardial infarction in mice
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Background and Aims: Enhanced metalloproteinase (MMP) activity is thought to be one of the major determinants of post-infarction LV remodelling. Former studies revealed MMP activation and development of HF in animals with very high glucose levels. The chronic effects of moderately elevated blood glucose on cardiac muscle are unknown.
The aim of the study was to assess the effects of long-term moderate streptozotocin (STZ) induced diabetes on MMP activity and LV remodelling following experimental MI in mice.
Materials and Methods: Moderate hyperglycaemia (MH) (200–400 mg/dL) was produced in 45 10–12 week old male C57Bl6/J mice by 5 injections of STZ (45 mg/kg); 42 age, sex and strain matched mice served as controls (C). After 8 weeks mice were subjected to left coronary artery ligation (MI) (n=61) or sham operation (SH) (n=26). Early infarct size was measured using Evans blue and TTC, 5 hours after MI. MMP activity was measured 5 days after MI or SH using gelatin zymography. Other mice were sacrificed 8 weeks post MI. Apoptosis assessment (TUNEL), trichrome staining and morphometric measurements of LV were performed.
Results: Staining with Evans blue and TTC revealed a comparable size of infarction in both groups. Five days after MI there was a similarly strong induction of MMP-2 and MMP-9 activity in infarct zone in all animals.
An increased activity of MMP-1 in infarct area only in C MI animals was observed. Increased MMP-9 activity in the

remote myocardium was observed only in MH MI and MH SH mice, but not in C animals. MH mice presented similar mortality during 8 weeks as controls. Morphometric parameters, apoptosis and fibrosis in viable myocardium after MI were significantly increased in comparison to SH animals, but there were no differences between MH and C mice, neither in MI nor SH groups.

Conclusion: These data suggest that prolonged moderate hyperglyceamia in mice affects MMP activity early after MI, but unlike in high hyperglycaemia, this effect is probably too modest to affect long term mortality and LV remodelling.

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Right ventricular diastolic function abnormalities in patients with type 1 diabetes mellitus

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Background and Aims: Type I diabetes mellitus (DM) has been associated with left ventricular diastolic dysfunction even before the development of signs and symptoms of heart disease. The aim of the study was the early detection of right diastolic dysfunction in asymptomatic patients with DM type I by the use of tissue Doppler imaging (TDI) indices.

Materials and Methods: We examined 45 patients (24 men, mean age 39,2±9,9), with DM type I (Group A) and 31 normal controls (Group B) of comparable age and sex. All the participants underwent a standard 2-D and color Doppler echocardiographic study. Pulsed TDI was performed in apical 4- chamber view.

We measured the LV ejection fraction (EF), transmittal flow velocity (E, A, E/A) and Tran tricuspid flow velocity (E′, A′, E′/A′). Additionally TDI measurements of mitral (mitral Em, Am, Sm) and tricuspid annulus velocities (tricuspid Em, Am, Sm) were evaluated. A possible correlation was examined for age, diabetes duration and echocardiographic measurements of left and right ventricle with univariate analysis.

The volume of right atrium, RA volume was calculated from the apical 4-chamber view using Simpson's method and consequently indexed to body surface area (RAVI).

Results: All the participants had normal ejection fraction, dimensions and wall thickness of the ventricle. Patients of the group A had impaired right diastolic function compared to the controls of group B.

	Diabetic patients	Controls	P
EF%	68±5	66±5	NS
E/A	0.95±0.6	1.8±5	<0.0001
E"/A"	0.94±0.58	1.77±0.48	<0.001
Mitral Sm cm/sec	6.1±0.9	6.2±0.75	NS
Mitral Em/Am	0.9±0.2	1.6±0.7	<0.0001
Tricuspid Sm	9.8±1.1	10.1±1.1	NS
Tricuspid Em/Am	0.89±0.39	1.38±0.38	<0.001
IVCv cm/sec	8.2±1.8	9.1±1.6	NS
Right atrium ml/m^2	15±5	14±3	NS

Conclusion: Asymptomatic patients with DM I have early right diastolic dysfunction that can be reliably detected using indices of tissue Doppler echocardiography.

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Endothelial dysfunction in patients with diabetes mellitus type 1 and diabetic cardiomyopathy

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Background and Aims: Diabetes influences myocardial and coronary vessels function by coexisting macroangiopathy, microangiopathy, metabolic disturbances and autonomic nervous system neuropathy. All these factors result in diastolic and systolic dysfunction of the heart. Subclinical diastolic dysfunction is served as a marker of a diabetic cardiomyopathy. The diabetic state is also associated with endothelial dysfunction as an initial manifestation of vascular abnormalities in diabetes. The aim of this study was to assess echocardiographic evidence of cardiomyopathy and its association with endothelial function in type 1 normotensive non-proteinuric diabetic patients.

Materials and Methods: Fifty patients with type 1 DM (mean age 30,1±1,8 years) and 20 healthy controls (mean age 29,9±1,8 years) were studied. Each patient was underwent echocardiography and assessment of brachial endothelial function. Indexes of left ventricular diastolic filling were measured by Doppler-echocardiography. Endothelial function was investigated using ultrasound assessment of flow-mediated dilatation (FMD). Data are presented as M±m. The results were compared using Student's paired test.

Results: No patient had chest pain or electrocardiographic changes during exercise testing. All patients had a normal ejection fraction (53,9±2,3 vs 56,1±4,3, ($p > 0,05$) in those with and without DM, respectively). A diagnosis of LVDD was made when at least one of the following parameters

was present in the echocardiographic study: isovolumetric relaxing time (IRT) >100 ms, deceleration time (DT) > 220 ms or early filling rate peak/late filling rate peak ratio (E/A)<1. According to these criterions 18 (36%) of the 50 diabetic patients had evidence of diastolic dysfunction as assessed by the presence of at least two abnormal variables of mitral inflow velocity. In particular, the ratio of peak early to peak late (atrial) filling velocity was significantly decreased in diabetic compared with control subjects (E/A ratio 0,9±0,05 vs 1,2±0,06, p<0,05), the DT was 257,1± 28,2 ms vs 186±14,6 ms p<0,05, in those with and without DM, respectively. Also the IRT was increased in diabetic patients compared with control subjects (130±14,0 ms vs 79,3±6,1 ms, p<0,05). The percent change in diameter of the brachial artery in response to reactive hyperemia was significantly impaired in patients with DM compared with control subject, and this impairment was significantly more prominent in patients with diabetic cardiomyopathy than in those without left ventricular diastolic dysfunction. According to our data, the percent change in diameter of the brachial artery in response to reactive hyperemia in patients with diabetic cardiomyopathy (8,4±1,5%) was significantly lower than that in healthy volunteers (17,6±1,3%), (p<0,05), and in patients with DM but without cardiomyopathy (13,6± 0,9%), (p<0,05)

Conclusion: Our data suggest that 1) more than one-third of young type 1 DM patients with a normal systolic ventricular function have diabetic cardiomyopathy; 2) impairment of endothelial function was significantly more prominent in patients with diabetic cardiomyopathy.

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Diabetic cardiomyopathy and endothelial dysfunction associated with microalbuminuria in patients with diabetes mellitus type 1

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Background and Aims: Diabetic cardiomyopathy (DMC) is a complication of diabetes mellitus (DM) that is more frequently observed in those patients with microalbuminuria. Left ventricular diastolic dysfunction (LVDD) in patients with diabetes, in absence of another etiology that justifies it, is an early marker of DMC. The diabetic state is also associated with endothelial dysfunction as an initial manifestation of vascular abnormalities in diabetes. On other hand, microalbuminuria is considered an important predictor of cardiovascular events in diabetic patients and as a marker of endothelial dysfunction. The aim of this study was to assess echocardiographic evidence of cardiomyopathy, endothelial dysfunction and their association with microalbuminuria in type 1 diabetic patients without hypertension, coronary artery disease, or autonomic dysfunction

Materials and Methods: Fifty young type 1 DM patients with LVDD, 30 with and 20 without microalbuminuria, and 20 control subjects were studied. Indexes of left ventricular diastolic filling were measured by Doppler-echocardiography. Endothelial function was investigated using ultrasound assessment of flow-mediated dilatation (FMD). Data are presented as M±m. The results were compared using Student's paired test.

Results: A diagnosis of LVDD was made when at least one of the following parameters was present in the echocardiographic study: isovolumetric relaxing time (IRT) >100 ms, deceleration time (DT) > 220 ms or early filling rate peak/ late filling rate peak ratio (E/A)<1. According to these criterions LVD function was significantly impaired in both diabetic groups compared with control subjects; moreover, this impairment was significantly greater in microalbuminuric than in normoalbuminuric patients. In particular, the ratio of peak early to peak late (atrial) filling velocity was significantly decreased in diabetic microalbuminuric patients compared with diabetic patients without microalbuminuria and control subjects (E/A ratio 0,7±0,05 vs 1,0± 0,06 vs 1,3±0,06, p<0,05), the DT was 257,1±28,2 ms vs 196±14,6 ms vs 176±14,6 ms p<0,05, in those with and without microalbuminuria and control group, respectively. Also the IRT was increased in patients with DM compared with control subjects (130±14,0 ms vs 79,3±6,1 ms, p<0,05,), but there were no significant differences between diabetic patients with and without microalbuminuria. The percent change in diameter of the brachial artery in response to reactive hyperemia in patients with LVDD and microalbuminuria (6,4±1,3%) was significantly lower than that in control subjects (12,6±1,4%), (p<0,05), and in diabetic patients with LVDD but without microalbuminuria (8,6±1,3%), (p<0,05)

Conclusion: Our data suggest that microalbuminuria is associated with significant changes of cardiac diastolic function and endothelial dysfunction. Microalbuminuria may be an early biochemical marker of these abnormalities. Therefore, microalbuminuric patients should be considered to have a higher risk of cardiovascular complications.

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Diabetic retinopathy is associated with left ventricular diastolic dysfunction in young patients with long lasting diabetes mellitus type 1

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Background and Aims: Diabetic retinopathy in young patients may coexist with other organs complications, especially subclinical disorders of cardiac function. Echocardiographic doppler evaluation (ECHO) of mitral valve flow (MVF) and pulmonary venous flow (PVF) is a good method for assessment of left ventricular diastolic function (LVDF). The aim of the study was to evaluate the LVDF using ECHO in young patients with long lasting diabetes mellitus type 1 (DM1), with or without diabetic retinopathy.
Materials and Methods: 65 patients (pts) with DM1 (33F, 32 M, aged 29.8±6.9 yrs, mean DM1 duration 16.3± 7.6 yrs) without evidence of cardiovascular disease were included in the study. Mean HbA1c level was 9.7±2.1%. Twenty nine patients (14 F, 15M) with diabetic retinopathy only (non-proliferative or proliferative) completed group I and 36 pts (19F, 17M) without diabetic retino-, nephro- and peripheral neuropathy formed group II. The control group C consisted of 30 healthy persons (15F, 15M). To assess LVDF, the MVF and PVF parameters were evaluated. In MVF, the mean value of the maximal velocity (Vmax) of early phase E to Vmax of late phase A ratio (E/A) was measured. In PVF the mean value of Vmax of diastolic flow (D) and Vmax of systolic flow (S) were assessed and the S/D ratio was calculated.
Results:

MVF and PVF results

Parameter	Group I	Group II	Group C	1. I vs II; 2. I vs C; 3. II vs C
E/A	1.18±0.2	1.42±0.3	1.54±0.3	1. p=0,003; 2. p<0,0001; 3. NS
S/D	1.22±0.2	1.06±0.1	0.9±0.1	1. p=0,005; 2. p<0,0001; 3. p<0,0001

The changes in MVF and PVF parameters (decreased E/A ratio and increased S/D ratio) suggest preclinical impairment of LV relaxation in diabetic patients, more pronounced in persons with diabetic retinopathy. There was no correlation between ECHO parameters and DM1 duration and metabolic control of diabetes.
Conclusion: The echocardiographic assessment of MVF and PVF shows that young asymptomatic patients with long lasting DM1 and diabetic retinopathy have more pronounced LV diastolic dysfunction than patients without diabetic complications.

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High visceral fat accumulation in subjects with non-ischaemic dilated cardiomyopathy

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Background and Aims: Preferential visceral fat (VF) accumulation, dyslipidemia and alterations in glucose homeostasis are well known risk factors for cardiovascular diseases, but less is known in dilated cardiomyopathy (DCM), especially in the early stages of the disease. Most of the studies were conducted in patients with coronary artery disease (CAD), where the co-existence of other abnormalities confounds the relationship between metabolic variables and left ventricular (LV) function. Thus, the aim of this study was to investigate the relationship between metabolic profile, VF and cardiac function/dimension in subjects with idiopathic DCM.
Materials and Methods: We recruited 30 male non-obese subjects, 16 with previously known non-ischemic DCM (age 56±3, BMI 25.9±0.8, EF 37±3%) and 14 controls (CT; age 50±1, BMI 26.9±0.4, EF=66±2%). Exclusion criteria were known diabetes or FPG>126 mg/dl. All subjects received a 3 h 75 g-OGTT for determination of glucose tolerance and 1.5 T MRI was used to measure VF, subcutaneous fat (SC) and cardiac function (i.e., left ventricular mass, LV-EDMi; left-ventricular end-diastolic volume, LV-EDVi and quantitative ejection fraction, EF). In all subjects BNP was determined as well.
Results: Compared to CT, DCM subjects had larger VF depot (122±16 vs 86±5 cm^2, p=0.04) and increased VF/SC ratio (0.68±0.08 vs 0.39±0.0, p=0.0024) despite similar BMI. All subjects had similar FPG (102±2 mg/dl, p=ns) and fasting plasma insulin (FPI; 13±1 μU/ml, p=ns) but DCM had increased trygliceride (TG; 124 [78] vs 83 [51] mg/dl), and TG/HDL (1.6±0.2 vs 0.9±0.1). After OGTT in CT group 5 subjects were found IGT, but none with type 2 diabetes (T2DM) whereas in DCM group 5 subjects were found IGT and 5 with T2DM. Regarding the cardiac parameters, as expected, DCM had higher values of LV-EDVi (127±9 vs 74±4 ml/m^2, p=0.0002), LV-EDMi (105±6 vs 73±4 g/m^2,p<0.0001) and BNP (26 [88] vs 7 [7] pg/ml).
Conclusion: Preferential VF accumulation is present in early stages of DCM and may represent a risk factor for the development of non-ischemic dilated cardiomiopathy.

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Impact of anaemia on the left ventricular structure in type 2 diabetic patients with advanced phase of nephropathy

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Background and Aims: The excess mortality in patients with diabetic end-stage renal disease (ESRD) is mainly due to cardiovascular events. Left ventricular (LV) hypertrophy is an independent risk factor for myocardial ischaemia, cardiac arrhythmia, sudden death, and heart failure. Chronic anaemia may also lead to adverse cardiovascular consequence. Patients with diabetic ESRD increase rates of LV hypertrophy and anaemia. The aim of this study is to evaluate the impact of anaemia on LV remodeling in type 2 diabetic patients with advanced phase of nephropathy.
Subjects and Method: M-mode echocardiography was performed in consecutive type 2 diabetic patients with nephropathy, whose serum creatinine concentration was 2.0 mg/dl and more, by one experienced examiner as a base-line (B). During follow-up period, echocardiography was performed 50 type 2 diabetic patients with nephropathy (39 men, age (mean(SD)) 58 (12) years) who did not have overt cardiac diseases (myocardial infarction, valvular failure, hypertrophic cardiomyopathy) at the point of a doubling of the base-line serum creatinine concentration (D). LV remodeling was defined by 4 categories according to LV mass index and relative wall thickness; normal (N), concentric remodeling (CR), concentric hypertrophy (CH), and eccentric hypertrophy (EH). Arterial blood pressure, HbA1c, haemoglobin, and traditional clinical risk factors of CVD were measured at both B and D points.
Results: The median duration of follow up was 451(56 – 1631) days. The change of serum creatinine was from 3.8 (1.1) at B point to 7.3(2.0) mg/dl at D point, systolic blood pressure from 151(19) to 152(19) mmHg, and diastolic blood pressure from 82(10) to 76(14) mmHg, respectively. In terms of LV remodeling during B and D point, LV hypertrophy increased as following the progress of diabetic nephropathy, the ratio of N(12 to 12%), CR(12 to 0%), CH (34 to 38%), and EH (42 to 50%), respectively. In those whose LV hypertrophy decreased (7 men, 2 women), haemoglobin level significantly increased from 8.8(0.7)% at B point to 10.0(1.8)% at D point (p=0.002), whereas HbA1c, arterial blood pressure, and serum lipid did not change significantly. In those whose LV hypertrophy increased (10 men, 5 women), haemoglobin did not change significantly from 9.5(0.8)% at B point to 8.7(1.5)% at D point (N.S.). There were no differences in age, HbA1c, arterial blood pressure, serum creatinine level, and serum lipid at B point between both groups.
Conclusions: In type 2 diabetic patients with advanced phase of nephropathy, the ratio of LV hypertrophy increases as following the progress of renal insufficiency. Improvement of anaemia during advanced phase of diabetic nephropathy is important for the prevention of LV hypertrophy in type 2 diabetic patients.

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Analogue compared to human insulin therapy: effects on metabolism and cardiac function in patients with type 2 diabetes and an intensive conventional insulin therapy (ICD)

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Background and Aims: Compared to human insulins (HI), analogue insulins (AI) offer the option of optimizing metabolism also in type 2 diabetes (T2D). Especially, fast acting insulin analogues lower postprandial glucose levels more effectively than human regular insulin. However, it is not known whether therapy with AI can also improve the subclinically impaired myocardial function in T2D. This prospective, randomized, open long term study compared the effects of ICD with AI vs. HI on metabolic control and systolic and diastolic myocardial function.
Materials and Methods: Inclusion criteria: T2D with preceding insulin therapy > 1 year. Goup 1: AI (insulin detemir+insulin aspart) and group 2: HI (NPH-insulin and regular human insulin). Systolic (Vs) and diastolic myocardial function (Ve) were assessed by tissue Doppler before and two hours after a standardized breakfast (48 g carbohydrates). Both treatment groups were comparable with regard to demographics, cardiac function and metabolic control at baseline. This interim analysis includes data from 60 patients after a 12-month treatment period.
Results: After 12 months, HbA1c did not change in both groups (in the AI group: baseline 6.7±1.1, endpoint 6.4± 0.9% (p<0.19); HI group: baseline 6.5±0.8, endpoint 6.5± 0.9%. Postprandial serum glucose was reduced (p<0.02) by 41±70 mg/dl in the AI group and by 0±44 mg/dl in the HI group (p<0.04 compared to AI). Fasting serum glucose was reduced by 17±44 mg/dl in the AI group and by 15±44 mg/dl in HI group. In the AI group, Vs increased significantly from 7.4±1.0 to 7.8±1.1 cm/s (p<0.03) as did Ve (7.7±1.1 to 8.4±1.6 cm/s, p<0.03) associated with parellel changes in the postprandial state (p<0.02). In the HI group, however, Vs (baseline 7.5±1.1, endpoint 7.5±0.9 cm/s) and Ve

(baseline 7.6±1.3, endpoint 7.9±1.7 cm/s) did not change in the fasting state and in the postprandial state.
Conclusion: Twelve months ICD-therapy with AI significantly improved postprandial glucose control and cardiac function compared to therapy with HI. The association of lower postprandial glucose levels and improved myocardial function should be taken into consideration when selecting an insulin regimen for people with T2D.
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Optimisation of glycaemic control by insulin pump therapy improves left ventricular contractility in type 1 diabetes

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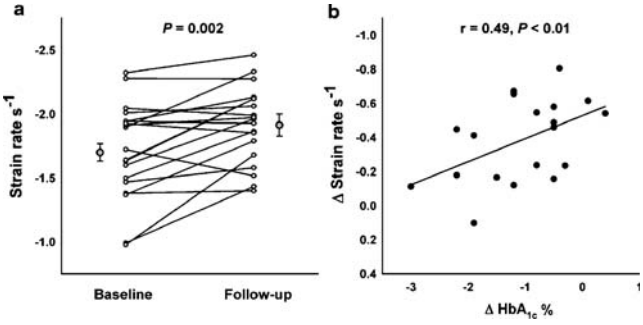
Decreased left ventricular long axis contractility may be the earliest stage in sub-clinical heart failure in type 1 diabetes. The aim of the study was to examine whether optimized glycemic control by insulin pump therapy would improve left ventricular contractility in a group of type 1 diabetic patients.
To assess whether improved blood glucose regulation, by intensified insulin treatment, would influence the left ventricular function, we included 19 consecutive type 1 diabetic patients selected for insulin pump therapy due to poor metabolic control. The main outcome measures were left ventricular strain rate in the long axis plane, examined by tissue doppler echocardiography and changes in Hba1c. Mean follow-up was 45 days (range 35–50). All patients included had an ejection fraction > 0.55% and fractional shortening > 0.25% and were free of any cardiac symptoms.
The mean age of the participants was 40 years (range 22–65). Following initiation of insulin pump therapy mean HbA1c was significantly improved from 8.6±1.4% to 7.6 ±1.1% ($p<0.01$). Mean strain rate of 16 myocardial segments was also significantly improved and decreased from $-1.58\pm0.30\ s^{-1}$ to $-1.80\pm0.4\ s^{-1}$ ($p<0.01$, figure A). The changes in left ventricular function were significantly correlated to the changes in HbA1c (delta-Strain rate vs. delta Hba1c: $r=0.49$, $p<0.01$ (figure.B). The changes in left ventricular function were more pronounced in the youngest part of the population (age at baseline vs. delta-Strain rate $r=-0.47$, $p<0.05$).
In conclusion, optimized glycemic control by insulin pump therapy improves left ventricular long axis contractility, mainly in young patients with type 1 diabetes.



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Peripheral endothelial function

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Endothelial barrier protective effect of FTY720 under the hyperglycaemic condition: involvement of focal adhesion kinase, small GTPases and adherens junction proteins

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Background and Aims: Sphingosine 1-phosphate (S1P) is a remarkably effective endothelial cell agonist that induces proliferation, calcium mobilization, adhesion molecule expression, and suppression of apoptosis. Recently, the role of S1P as an endothelial barrier stabilizing mediator has been highlighted. FTY720 is a S1P analog originally developed as a novel immunosuppressant. Phosphorylated form of FTY720 binds to S1P receptors to exert S1P-like biological effects, suggesting endothelial barrier promotion by FTY720. These lines of evidences give raise a question if FTY720 ameliorates diabetic angiopathy, the crucial cause of several diabetic complications, via vascular endothelial barrier enhancement. In the present study, we investigated the role of FTY720 on focal adhesion (FA) and adherens junction (AJ) remodeling associated with actin redistribution under both normoglycemic and hyperglycemic conditions.
Materials and Methods: Human microvascular endothelial cells (HMVECs) were preincubated with hyperglycemic (30 mM) media for 48 h and then treated with 100 nM FTY720 for 3 h. To monitor effects of hyperglycemic challenge and FTY720 treatment on the distribution of focal adhesion kinase (FAK), α-catenin, β-catenin and VE-cadherin, immunfluorescent microscopy was performed. To investigate the possible effect of FTY720 on FA and AJ

remodeling, β-catenin was immunoprecipitated and then, amounts of coprecipitated VE-cadherin and FAK was evaluated. Activity of small GTPase Rac and Rho and site-specific FAK tyrosine-phosphorylation were also monitored.
Results: Immunfluorescent microscopy and coprecipitation study revealed the FTY720-induced FAK-associated AJ assembly at cell-cell contacts coincident with the formation of a prominent cortical actin ring. Similar to S1P, site-specific FAK tyrosine-phosphorylation analysis revealed FTY720-induced FAK [Y^{576}] phosphortylation without phosphorylation of FAK [Y^{397}/Y^{925}]. Furthermore, FTY720 conditioned the phosphorylation profile of FAK [$Y^{397}/Y^{576}/Y^{925}$] in hyperglycemic media to the same pattern observed in normoglycemic media. FTY720 challenge resulted in small GTPase Rac activation under hyperglycemic condition, whereas increased Rho activity in hyperglycemic media was restored to the basal level.
Conclusion: We investigated the role of FTY720 on FA and AJ remodeling in HMVECs under both normoglycemic and hyperglycemic conditions. These results strongly suggest the pivotal role of FTY720 to ameliorate endothelial barrier function disturbed by hyperglycemic challenge, further implying the possibility of FTY720 as a therapeutic agent of diabetic vascular disorder.
This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Culture, Sports, and Technology of Japan.

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Protective role for AMPK-activated protein kinase in palmitate-induced HUVEC apoptosis

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Background and Aims: Vascular diseases are the principal cause of morbidity and mortality in patients with diabetes. While high plasma free fatty acid levels are associated with atherosclerosis and vascular complication, the mechanism of vascular endothelial apoptosis by palmitate is usually obscured. Therefore, we have studied the role of AMP-activated protein kinase (AMPK) on the death of palmitate induced human umbilical vein endothelial cells (HUVEC).
Materials and Methods: Palmitate induced HUVEC death was measured by DNA ladder, DAPI and MTT assay. To determine the mechanism of HUVEC death by palmitate, we used several inhibitors and AMPK adenovirus
Results: Treatment of palmitate increased HUVEC cell death in a dose and time-dependent fashion. Also palmitate increased the activity of caspase 9, 3 and that of DNA ladder. In investigating the mechanism of palmitate induced cell death, HUVEC were simultaneously incubated with palmitate and several inhibitors, SP600125 (JNK inhibitor), SB203580 (p38 inhibitor), GO6983 (PKC inhibitor), fumonisin B1 (ceramide synthesis inhibitor), NAC (ROS scavenger) and AICAR (AMPK activator). The degree of apoptosis was then measured. The preventive effects of NAC, SP600125 and SB203580 were 30%, 30% and 40% respectively. GO6983 and fumonisin B1 were 3% and 10%, respectively. AICAR and overexpression of constitutively active AMPK completely blocked palmitate induced HUVEC death.
Conclusion: In this study, palmitate induced HUVEC apoptosis was prevented by AMPK activation. So we think that vascular endothelial apoptosis of palmitate is caused by accumulation of fatty acid through fat synthesis metabolism.
This research was supported by the "GRRC" Project of Gyeonggi Provincial Government, Republic of Korea

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Acute hyperhomocysteinaemia blunts endothelial dependent and endothelial independent vasodilatation in diabetic patients

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Background and Aims: Homocysteine (Hcy) is believed to cause vascular damage by interfering with endothelial function, platelet activation, oxidative stress, hypercoagulability and vascular smooth muscle cell proliferation. Diabetic persons are known to have endothelial dysfunction and a very high incidence of atherosclerosis. The aim of this study was to investigate the effect of acute hyperhomocysteinemia on endothelial function of skin microcirculation in diabetic persons, compared to non-diabetic ones.
Materials and Methods: We compared 20 type 2 diabetic persons (59.95±10.54 years old, 10 males-10 females, diabetes duration 7.7±5.1 years) with 24 non-diabetic ones (58.42±10.93 years old, 7 males-17 females). Endothelial and non-endothelial dependent vasodilatation of the skin microcirculation was evaluated with the iontophoretic administration of acetylcholine (Ach) and sodium nitroprusside (SNP) respectively, on the forearm, during fasting

and 4 hours after 0.1 mg/kg methionine-induced acute hyperhomocysteinemia.
Results: The two groups were comparable regarding age and the other biochemical characteristics, with the exception of body mass index, waist circumference, fasting glucose, HbA1c, HOMA resistance index, fibrinogen and uric acid (higher in diabetics) and HDL-cholesterol (lower in diabetics). Both endothelial-dependent vasodilatation (Ach effect) and endothelial-independent vasodilatation (SNP effect) were significantly lower in the diabetic patients compared to the control group, after induction of acute hyperhomocysteinemia (p=0.027 and p=0.08, respectively).
Conclusion: Acute hyperhomocysteinemia causes blunting of endothelial-dependent and endothelial-independent vasodilatation of the skin microcirculation in diabetic persons, compared to non-diabetic ones. This may contribute to the atherogenic potential of Hcy.

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Insulin-like growth factor-binding protein-related protein 1 (IGFBP-rP1/MAC25) contributes to endothelium-dependent vasodilation in high-ferritin type-2 diabetes

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Background and Aims: Blood letting improves vascular reactivity in high ferritin type-2 diabetic subjects (T2DM). Insulin-like growth factor (IGF)-binding protein-related protein 1 (IGFBP-rP1; also known as mac25 and angiomodulin) is a novel vascular factor involved in oxidative stress whose role in T2DM is largely unknown. We hypothesized that circulating IGFBP-rP1 modulates the vascular changes that follow iron depletion in T2DM.
Materials and Methods: Twenty-six T2DM male patients with high ferritin levels (>200 ng/ml) randomized to either blood letting (three extractions of 500 ml of blood at two-week intervals; n=11) or to observation (n=15) were studied. Endothelium-dependent and independent vasodilation were measured in the brachial artery by high-resolution ultrasound at baseline and at 4 months thereafter. The groups were matched for age, BMI, pharmacological treatment, chronic diabetes complication and baseline serum IGFBP-rP1.
Results: Circulating IGFBP-rP1 was significantly associated with endothelium-dependent, but not endothelium-independent vasodilation at baseline in these subjects (studied as a single group; r=0.49, *P*=0.014). Although blood letting did not have a significant effect on circulating IGFBP-rP1 [mean (SEM) for percentage change at 4 months:+39% (13%) *vs.*+25% (13%), for intervention and observation groups, respectively; *P*=0.44], significant correlations were documented between the changes in endothelium-dependent vasodilation at 4 months and both baseline serum IGFBP-rP1 (r=−0.48, *P*=0.016) and its change during this time (r=0.56, *P*=0.008). Finally, both endothelium-dependent vasodilation and circulating IGFBP-rP1 were significantly correlated with plasma cyclic GMP in these subjects (r=0.51, P=0.04 and r=0.68, *P*=0.003, respectively).
Conclusion: These results suggest for the first time that IGFBP-rP1 modulates endothelium-dependent vasodilation in T2DM. The contribution of IGFBP-rP1 to the vascular dysfunction of diabetes mellitus merits further research.
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Vascular endothelial dysfunction evidenced by decreased flow mediated dilation is an early manifestation in subjects with fasting euglycaemia but impaired 2-hour glucose levels

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Background and Aims: Increased prevalence of hyperglycaemia in the general population is largely manifested as elevated 2 hour post-challenge glucose concentrations. Normal fasting glucose depends on adequate basal insulin secretion and sensitivity, mainly in the liver, in order to control glucose output, whereas impaired 2 hour plasma glucose levels after an OGTT reflect peripheral insulin resistance, particularly in skeletal muscle, the main site for glucose disposal. We investigated the potential effect of postchallenge hyperglycemia on endothelial dysfunction as an early manifestation of vascular disease in subjects with fasting euglycemia.

Materials and Methods: We carried out an OGTT for 2 hours with two blood samples per subject (baseline and 2 hours post-glucose (75 grams) ingestion), on 38 age- and BMI- matched participants (age 49±2 vs 51±3 yrs and BMI 28±1 vs 29±1, respectively), who all had normal fasting plasma glucose levels (<100 mg/dl). Group A subjects (N=24) had normal glucose tolerance (2 h-glucose<140 mg/dl; 91±5 mg/dl) whereas Group B subjects (N=14) exhibited impaired 2 h plasma glucose levels (165±7 mg/dl). FMD of the brachial artery was determined by high resolution ultrasonography as a surrogate marker of endothelial function before OGTT was performed.
Results: Plasma insulin, total-, HDL- and LDL cholesterol, triglycerides and the HOMA-IR insulin resistance index did not differ between subject groups. Similarly, glycosylated hemoglobin (HbA_1c) was not significantly different between groups (4.8±0.5 vs 5.0±0.1%, respectively). However FMD was significantly lower in subjects with impaired 2 h glucose compared to those with normal glucose tolerance (2.6±0.8 vs 7.9±0.6%; p<0.001).
Conclusion: In otherwise healthy non-obese individuals with fasting euglycemia, the presence of glucose intolerance after glucose ingestion was associated with diminished FMD compared to the normal glucose tolerant state. This finding could be of particular significance to the early pathophysiology of vascular endothelial dysfunction observed in insulin resistant states such as the metabolic syndrome and demonstrates the importance of optimal glycemic control on the maintenance of normal vascular function.

1199

Rosiglitazone restores endothelial function in Zucker Diabetic Fatty rats

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Background and Aims: Obesity and type 2 diabetes are associated with endothelial dysfunction. The aim of the study was to assess for the first time in Zucker Diabetic Fatty (ZDF) rats, a model of type 2 diabetes, if the insulin sensitizer rosiglitazone may reduce endothelial dysfunction, as well as vascular reactivity to insulin and blood pressure abnormalities.
Materials and Methods: Three series of 7–9 rats were studied at 24 weeks of age: Lean (fa/?) controls, ZDF (Gmi-fa/fa) rats treated with rosiglitazone (7 μmol/kg/day) for 16 weeks (ZDF-ROS) and untreated ZDF rats. Central aortic pressure was measured in anesthetised rats at the end of the treatment. Thoracic aorta was removed and the dose-response curves to insulin (30–10^5 mIU/L) and acetylcholine (Ach:$10^{-10}-10^{-4}$ M) were studied *in vitro* after a precontraction with Norepinephrine (10^{-6} M), in the presence or absence of N-Nitroω-L-Arginine (L-NNA 3.10^{-5} M) or indomethacin (IND 3.10^{-5} M).
Results: Body weight was increased in ZDF-ROS rats (510±47 g) as compared to ZDF rats (379±8 g, p<0.01) and to controls (429±9 g, NS). In ZDF-ROS rats, systolic blood pressure (SBP: 160±5 mmHg) and pulse pressure (PP: 51±4 mmHg) were increased as compared to untreated ZDF rats (146±2 mmHg, p<0.05; 39±1 mmHg, p<0.01, respectively) and control rats (138±6 mmHg, p<0.0001; 31±2 mmHg, p<0.0001, respectively). The vasorelaxation response to insulin observed in control rats was inhibited by L-NNA, was absent in untreated ZDF rats and was partially restored in the ZDF-ROS rats. Impaired Ach-induced relaxation in ZDF rats compared to controls was also partially restored in ZDF-ROS rats. L-NNA but not IND blocked Ach-induced relaxation.
Conclusions: Rosiglitazone, despite weight gain, higher SBP and PP that occur here in contrast to human data, restores endothelium-dependent relaxation induced by insulin and Ach in the thoracic aorta of this model of type 2 diabetes.
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1200

The effect of leucine consumption on endothelial function, plasma glucose and insulin levels in healthy male subjects

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Background: It is known that leucine consumption enhances insulin secretion. Data on the effect of acute hyperglycaemia on endothelial function in healthy subjects are controversial. Recent data showed that hyperinsulinaemia may exert a favourable effect on endothelial function. The combined, however, effect of hyperglycaemia and hyperinsulinamia on endothelial function has not been examined so far. This study examined the potential differential effect of hyperglycaemia and hyperinsulinaemia induced by glucose infusion alone and in combination with leucine consumption on endothelial function in healthy subjects.
Subjects-Methods: Ten healthy, non-obese males (mean age 31.8±5.5 years) were studied twice. In one visit, they consumed 250 ml of water (placebo) p.o. at baseline and 30 min later glucose 35% (0.5 g/kg of body weight) was infused i.v. (phase A). In the other visit, leucine 25 g in

250 ml water p.o.was consumed at baseline and 30 min later the same amount of glucose was infused (phase B). Endothelial function was evaluated by measurement of flow-mediated dilatation (FMD) using high resolution ultrasonography at baseline, 40 min and 90 min afterwards. Plasma glucose and insulin levels were measured at baseline and every 10 min after glucose infusion.

Results: At 40 min of the phase A, FMD decreased significantly-compared with the baseline values- by 73.7% (median value, P=0.006); the respective value in phase B was+8.2%, P=0.80). At 90 min, there were no significant differences in FMD change after either experiment. Plasma glucose levels increased significantly and to the same degree at all time intervals studied after both experiments. Insulin levels increased much more during phase B as compared to phase A.

Conclusions: In healthy male subjects, acute hyperglycaemia impairs endothelial function. A concomitant, however, increase in plasma insulin levels after combined glucose - leucine consumption exerts a protective effect on endothelial function.

		0 min	40 min	50 min	60 min	70 min	80 min	90 min
Glucose +placebo (Phase A)	Glucose (mg/dl)	90.0 ±7.9	340.9 ±40.0	245.1 ±25.9	187.5 ±42.2	144.2 ±49.2	117.4 ±42.6	100.7 ±31.1
	Insulin (μIU/ml)	11.5 ±1.7	55.2 ±25.7	60.4 ±38.1	42.8 ±19.7	37.0 ±19.0	27.3 ±12.5	21.3 ±9.1
Glucose +leucine (Phase B)	Glucose (mg/dl)	89 ±12.4	339.6 ±50.2	237.7 ±62.0	182.6 ±63.2	140.4 ±58.6	105.9 ±38.7	85.1 ±31.4
	Insulin (μIU/ml)	13.6 ±7.6	142.9 ±92.7	166 ±79.0	122.8 ±49.4	88.5 ±35.0	60.0 ±24.5	42.2 ±19.0

1201

Effects of 17-β estradiol treatment on vascular reactivity of ovariectomized and diabetic rats

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Background and Aims: Menopause is an independent risk factor for cardiovascular diseases and it's believed that diabetes contributes this situation. Estrogen replacement therapy is associated with reduction in cardiovascular mortality in postmenopausal women. The aim of the present study was to show if there is any effect of 17-β estradiol (E2) on regulation of responsiveness of aortae from both diabetic (Dia) and non-diabetic ovariectomized (OVX) rats.

Materials and Methods: 4 groups have been used in this study: OVX (n=8), OVX+E2 (n=8), Dia+OVX (n=8) and Dia+OVX+E2 (n=10). Bilateral ovariectomy was applied to anesthetized animals and diabetes induced by a single i.p. injection of streptozotocin (STZ; 45 mg/kg) a week after the operation. E2 treatment has been started on the 3rd day of the operation (1 mg/kg day, orally). After 8 weeks blood glucose (BG), total cholesterol (CH) and triglyceride (TG) concentrations have been measured as final values. Blood pressure (BP) levels have been measured by non-invasive tail cuff method weekly. Abdominal aortae have been used for functional studies in organ baths. KCl (10–60 mM) and phenylephrine (PE; 10^{-8}–10^{-4} M) were used in cumulative concentrations as contractile agents. In order to show functional endothelium, aortae were pre-contracted with submaximal concentration of PE (10^{-6} M) and then acethylcholine (ACh; 10^{-6} M) was applied to the aortae. Incubations with L-Nitro-L-Methyl Arginine Methyl Ester (L-NAME; 10^{-6} M), superoxide dismutase (SOD; 10^{-6} M) and indomethacine (INDO; 10^{-6} M) were performed to compare the magnitudes of PE-induced submaximal contractions and ACh-induced relaxations (10^{-6} M).

Results: Although the BG concentration was not affected by the treatment, TG and CH concentrations were significantly normalized (P<0.05 vs. OVX+Dia). E2 treatment also reduced the BP values in Dia+OVX and OVX groups (P<0.05 vs. OVX+E2). KCl- and PE-induced contractions were found that increased in both Dia and Dia+OVX aortae and reduced by the treatment (P<0.05 vs. OVX+E2).The contractile effect of PE was increased by L-NAME, and reduced by SOD and INDO in all aortic groups. The vasodilator effect of ACh was increased by SOD and INDO, and reduced by L-NAME in all aortic groups.

Conclusion: These results suggests that E2 treatment has some beneficial effect on vascular reactivity in both diabetic and non-diabetic OVX animals and this effect may partly due to the antioxidant property of E2 itself.

% Changes on PE-induced (10–6 M) Contractions

	L-NAME	SOD	INDO
OVX	40.18±0.12*# ↑	46.36±1.34*# ↓	47.53±0.46* ↓
OVX+E2	64.60±0.22 ↑	21.41±1.02# ↓	40.37±0.68‡ ↓
OVX+DiA	41.60±0.18*# ↑	48.00±0.63*# ↓	45.03±1.14† ↓
OVX+DiA+E2	68.10±0.10 ↑	11.30±1.32* ↓	44.93±1.11† ↓

% Changes on ACh-induced (10-6 M) Relaxations

	L-NAME	SOD	INDO
OVX	77.38±1.54*# ↓	29.10±1.32*# ↑	9.57±0.65†‡ ↑
OVX+E2	88.35±1.62 ↓	22.10±1.74 ↑	11.46±0.21 ↑
OVX+Dia	79.33±1.77*# ↓	32.26±1.12*# ↑	9.08±0.16†‡ ↑
OVX+Dia+E2	84.35±1.83 ↓	18.40±1.22 ↑	11.47±0.37 ↑

*P<0.001 and †P<0.01 *vs.* OVX+E2
# P<0.001 and ‡P<0.01 *vs.* OVX+Dia+E2

1202

The effect of benfotiamine on endothelial function in patients with type 2 diabetes mellitus

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Background and Aims: Many studies show a strong relationship between hyperglycemia and diabetic microvascular complications, and hyperglycemia seems to have a important role in the pathogenesis of diabetic macrovascular disease. There are a number of potential strategies to pursue to prevent or possibly reverse damage caused by hyperglycemia. Typically, the most effective strategies would be aimed at the vasculature, which appears to be the first organ system to undergo hyperglycemia induced damage. Benfotiamine is a synthetic thiamine derivative that activates the enzyme transketolase to redirect glycolytic metabolites from vascular damaging pathways. Recently in diabetic animals benfotiamine treatment inhibited major pathways implicated in pathogenesis of hyperglycemia induced vascular damage and NF-kB activation. Endothelial dysfunction is earliest sign involved in the pathogenesis of diabetic complications.
Materials and Methods: We evaluated the change of plasma soluble E-selectin, ICAM-1 and VCAM-1 concentration after the administration of benfotiamine in patients with type 2 diabetes. Plasma soluble E-selectin, ICAM-1 and VCAM-1 (a marker of endothelial function) were measured using commercial ELISA kit. Eighteen type 2 diabetes patients (age 57.7±6.75 years, BMI 24.6±1.74 kg/m^2 and DM duration 13.1±5.64 years) were studied and benfotiamine 120 mg was administered twice daily for two months.
Results: 1. Plama E-selectin level was decreased significantly from 39.3±3.34 ng/mL to 34.8±2.60 ng/mL after administration of benfotiamine (P<0.05).
2. Plama ICAM-1 concentration was decreased significantly from 225.4±18.39 ng/mL to 205.1±16.03 ng/mL after administration of benfotiamine (P<0.05).
3. Plama VCAM-1 was decreased significantly also from 31.9±7.81 ng/mL to 26.6±5.07 ng/mL after administration of benfotiamine (P<0.05).
Conclusion: These results showed that benfotiamine can decrease plasma E-seletin, ICAM-1 and VCAM-1 level and improve endothelial function in patients with type 2 diabetes. So benfotimine might be clinically useful in preventing the development and progression of diabetic vascular complications.

1203

Endothelial progenitor cells are reduced but activated in type 1 diabetic patients with longstanding disease and high HbA_{1c}

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Background and Aims: Two recent european epidemiological studies indicate an excess mortality, mainly caused from cardiovascular disease (CVD), in patients with type 1 diabetes (T1DM) despite advances in diabetes care. Since a reduced number of endothelial progenitor cells (EPC) was recently found to predict CVD death and events in nondiabetic patients, our study was aimed to quantify EPC in well-characterised patients with T1DM.
Materials and Methods: 45 patients with T1DM (HbA_1c: 7.8±1.9%; age: 36±13 years; BMI: 24.5±3.4 kg/m^2) were studied in comparison with 30 age- and sex-matched control subjects. The T1DM patients were divided into three groups according to their duration of disease: <10, 10–25, and >25 years. Circulating progenitor cells (CPC, defined as CD34+/CD133+), endothelial progenitor cells (EPC, defined as CD34+/CD133+/CD309+) and activated EPC (actEPC, defined as CD34+/CD133+/CD309+/CD31+) were enumerated by flow cytometry. The EPC/CPC ratio as well as the actEPC/EPC ratio was determined. T1DM patients presenting with diabetic retinopathy were excluded from the study.
Results: CPC, EPC and actEPC were significantly reduced in T1DM patients in comparison with control subjects (CPC: 1264±421 vs 529±167, p<0.001; EPC: 365±164 vs 109±80, p<0.001; actEPC: 150±59 vs 62±48, p<0.001). Interestingly, the EPC/CPC ratio was lower in the T1DM compared with the controls (21±13% to 29±9%, p<0.001), whereas the actEPC/EPC ratio was increased in the T1DM versus controls (62±25% to 42±8 to p<0.001). By univariate regression analysis, age had the strongest association with the decline in CPC (r=−0.335, p=0.003), diabetes duration with the decline of EPC (r=−0.434, p<0.001), and

HbA1c showed the strongest association with the activation of EPC as detected by the adhesion molecule CD31 (HbA1c to actEPC/EPC ratio: r=0.380, p=0.001).
Conclusion: Type 1 diabetic patients with long duration of disease and high HbA1c have significantly less numbers of CPC and EPC. Remarkably, the rate of activated EPC as determined by expression of CD31 on EPC surface is elevated in these patients. The increase in activated EPC could be a compensatory response to augmented demand of vascular repair in those type 1 diabetic patients, who have longstanding disease, poor glycemic control and are at increased risk for cardiovascular morbidity and mortality.

PS 108

Arterial stiffness and remodelling

1204

Metabolic syndrome and intima media thickness - an observation of the RIAD-study
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Background and Aims: Several studies have shown that patients with metabolic syndrome have significant more cardiovascular events. Scarce information exist, how far the intima media thickness (IMT) of common carotid arteries as marker of early atherosclerotic changes was influenced by metabolic syndrome (MS).
Materials and Methods: A total of 566 middle-aged subjects participate on the Risk Factors in Impaired Glucose Tolerance for Atherosclerosis and Diabetes (RIAD) were classified as follows: 108 without traits of MS (group 1), 135 with single trait (group 2), 150 with two traits (group 3) and 173 with three or more traits (group 4) of MS. MS was defined by $NCEP_{III}$ ATP criteria. IMT was measured by high resolution ultrasound. Increased IMT was defined as the value over the 3rd percentile (≥ 0,95 mm).
Results: No difference in patients with increased IMT was found between group 2 and group 1. Patients with increased IMT were significant more frequent in group 3 and group 4 comparing with group 1 (OR 1,93 vs 1,72, p=0,012 vs p=0,032). No difference in patients with increased IMT was found between group 3 and group 4.
Conclusion: Increased IMT was significant more often in patients with MS (≥3 traits). These early atherosclerotic changes were not only seen in patients with MS but also in patients with "pre"metabolic syndrome (2 traits).

1205

Postprandial glucose is associated with carotid intima media thickness but postprandial triglycerides are not. The HOORN PRANDIAL STUDY
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Background and Aims: Both postprandial hyperglycemia (ppGL) and postprandial hypertriglyceridemia (ppTG) have been identified as stronger risk markers for atherosclerosis than the respective fasting levels. However, the relative contribution of ppGL and ppTG to atherosclerosis has not been investigated until now. The aim of this study was to compare the associations of ppGL and ppTG with carotid intima media thickness (IMT) following two consecutive high-fat meals or two consecutive high-carbohydrate meals on two separate days.
Materials and Methods: Non-smoking, post-menopausal women, aged 50–65 years were included; 76 women with normal glucose metabolism (NGM, WHO-99 criteria) and 78 women with type 2 diabetes (DM) of whom 37 were using statin medication. Blood samples were taken at both visits and GL and TG were measured before and at t=1, 2, 4, 6 and 8 hours following breakfast; lunch was given at t=4. The high-fat breakfast and lunch contained 3.3 MJ; 50 g fat and 56 g carbohydrates. The high-carbohydrate breakfast and lunch contained 3.2 MJ; 4 g fat and 162 g carbohydrates. IMT was measured by ultrasound imaging of the right common carotid artery at 10 mm proximal to the carotid bulb. Linear regression analysis was used to calculate associations with IMT, we tested for interaction with DM and use of statin medication.
Results: Mean IMT was 764 and 780 μm for women with NGM and DM, respectively. In the complete study population, age was statistically significantly associated with IMT (standardized regression coefficient (95% CI) in μm IMT was 20.2 (2.6;37.8)). In women with NGM, ppGL levels after both the high-fat and the high-carbohydrate meals were positively associated with IMT. Standardized regression coefficients (95% CI) in μm IMT were 92.6 (6.1;179.1) for ppGL after the high-fat meals and 98.8 (−13.7;211.3) for ppGL after the high-carbohydrate meals with adjustment for age (Table).

Associations (standardized beta (95% CI) in μm IMT, adjusted for age and use of statins)

	NGM (n=76)	DM (n=78)
Fasting TG	2.3 (−26.0;30.7)	−5.6 (−34.1;22.9)
Fasting GL	50.4 (−45.8;146.6)	**38.6 (6.2;71.0)#**
Fat meals		
PpTG	3.9 (−23.1;30.9)	−13.4 (−40.3;13.6)
PpGL	**92.6 (6.1;179.1)**	8.6 (−18.5;35.6)*
Carbohydrate meals		
PpTG	10.0 (−16.5;36.4)	−10.1 (39.6;19.5)
PpGL	98.8 (−13.7;211.3)	14.4 (−13.1;42.0)*

#Women using statins were excluded because of interaction with use of statins (p=0.07)
*Interaction with DM (p<0.15)

These associations were not explained by BMI, waist, blood pressure, total cholesterol, LDL-c or HDL-c. No association between ppGL and IMT in women with DM was found (p-value for interaction with DM p<0.15). PpTG were not associated with IMT in women with NGM nor in women with DM. In women with DM no interaction was found with use of statins, except for fasting GL (p=0.07).
Conclusion: The association between ppGL and IMT was stronger than that of ppTG and IMT. The association between ppGL and IMT in women with NGM suggests that ppGL even in the normal range, is a marker or a risk factor of CVD.
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1206

Arterial stiffness is inversely related to adiponectin levels in young, normotensive type 1 diabetic patients

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Background and Aims: Arterial stiffness, a contributing factor to cardiovascular morbidity and mortality, is increased even at the early stages of type 1 diabetes and is associated with the presence of cardiac autonomic neuropathy. Adiponectin is an adipose tissue derived adipokine that has recently attracted much attention as a posible insulin-sensitizing and antiatherogenic factor. Plasma adiponectin (ADPN) concentrations are low in individuals with insulin resistance such as those with type 2 diabetes. Only few studies have examined adiponectin levels in patients with type 1 diabetes and have consistently shown that these patients have increased plasma levels of that hormone. Recently it has been reported that increased adiponectin levels in patients with type 1 diabetes are prospectively associated with a lower risk of coronary artery disease.
Aim: The aim of this study was to investigate the association of arterial stiffness with ADPN levels and autonomic dysfunction in young, normotensive type 1 diabetic patients.
Materials and Methods: 43 normotensive type 1 diabetic patients, aged less than 40 years were consecutively selected from the outpatient diabetes clinic of our institution. Subjects on statin therapy as well as those with macrovascular disease or overt nephropathy were excluded. Large artery stiffness was assessed by measurement of carotid-femoral pulse wave velocity (PWV) and plasma ADPN was measured by radio-immuno assay. Autonomic function was estimated on the basis of the battery of classic cardiovascular autonomic function tests (Ewing tests).
Results: Data from 43 subjects were analyzed (mean age 26.8±5.9 years, body mass index [BMI] 24.1±2.9 kg/m^2, HbA_{1C} 7.5±1.3%, 20 [45.5%] men). Mean PWV and ADPN values were 5.5±0.9 m/s and 15.3±6.8 μg/mL, respectively. There was a significant positive correlation of PWV with age (r=0.508, p=0.001), duration of diabetes (r=0.278, p=0.048), BMI (r=0.303, p=0.048), waist circumference (r=0.448, p=0.003) and systolic blood pressure (r=0.375, p=0.013). Nevertheless, PWV showed a reciprocal relationship with inspiration/expiration index (r=−0.360, p=0.018) and plasma ADPN (r=−0.418, p=0.01). In multivariate stepwise linear regression analysis, PWV was independently associated to plasma ADPN (standardized β-regression coefficient=0.480, p=0.001), duration of diabetes (β=0.342, p=0.019) and systolic blood pressure (β=0.345, p=0.016). The R^2 of the statistical model was 0.468.
Conclusion: Arterial stiffness appears to be inversely related to plasma ADPN concentration in young, normotensive patients with type 1 diabetes without major complications, the latter association being independent of the presence of cardiac autonomic neuropathy. This novel finding suggests that the above adipocytokine might have a protective role against macrovascular disease in the population studied.

1207

High vitamin B6 is associated with lower carotid distensibility and compliance in type 2 diabetes but not in normal or impaired glucose metabolism: the HOORN STUDY

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Background and Aims: High homocysteine and low levels of vitamin B6 have been associated with cardiovascular disease. Our aim was to determine whether homocysteine (Hcy) and vitamin B6 are associated with carotid stiffness in people with and without type 2 diabetes.
Material and Methods: The study was performed among participants of the 2nd follow-up of the Hoorn Study, a population-based study of diabetes and cardiovascular disease. Data on Hcy and vitamin B6 and carotid stiffness was available for 545 people, 237 with normal glucose metabolism (NGM), 112 with impaired glucose metabolism (IGM) and 196 with type 2 diabetes (DM2), 50% men, mean age 68 years. Distensibility and compliance coefficients (DC and CC) of the right common carotid artery were obtained by a single observer with an ultrasound scanner with a 7.5 MHz linear probe (350 series, Pie Medical). Total fasting plasma homocysteine was determined on an Abbott Imx analyser and vitamin B6 with HPLC. Multiple linear regression analysis was used and age, sex and mean arterial pressure were included as standard correction variables.
Results: High Hcy was not associated with carotid stiffness in the various categories of glucose metabolism (see table). In contrast, high vitamin B6 was associated with lower carotid distensibility and compliance coefficients in patients with type 2 diabetes, but not in NGM or IGM. The association of high B6 in type 2 diabetes was independent of Hcy, folate, vitamin B12, prior CVD, current smoking or renal function.

	CC			DC		
	NGM	IGM	DM2	NGM	IGM	DM2
Hcy 2nd tertile	0.027	−0.042	−0.014	−0.260	−0.262	0.212
	(.412)	(.518)	(.630)	(.642)	(.793)	(.722)
Hcy 3rd tertile	0.044	−0.082	−0.015	0.133	−0.694	0.907
	(.186)	(.210)	(.066)	(.815)	(.488)	(.163)
B6 2nd tertile	−0.058	−0.046	−0.032	−0.537	−0.746	−0.411
	(.083)	(.409)	(.289)	(.359)	(.382)	(.485)
B6 3rd tertile	−0.056	0.035	**−0.112**	−0.070	0.504	**−1.891**
	(.086)	(.554)	**(.000)**	(.902)	(.578)	**(.002)**

Data are linear regression coefficients and (p-values)

Conclusion: In this cohort of elderly people high vitamin B6, but not Hcy, was associated with higher carotid stiffness in patients with type 2 diabetes but not in people with normal or impaired glucose metabolism.
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1208

Predictors of aortic stiffness in elderly type 2 diabetic patients in terms of diabetic nephropathy

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Background and Aims: Carotid-femoral pulse wave velocity (PWV) reflects arterial stiffness. Several studies demonstrate that increased PWV is a predictor of future cardiovascular events with diabetes mellitus. However, it is difficult to measure PWV of all diabetic patients because there are many numbers of diabetic patients. The aim of this study was to investigate the predictors of aortic stiffness in elderly type 2 diabetic patients in terms of diabetic nephropathy.
Materials and Methods: We studied cross-sectionally 128 elderly type 2 diabetic outpatients of 65 to 86 years of age [73.0+/−6.7 years(mean+/−SD), duration of diabetes 15.9+/−7.5 years]. All of the subjects had no evidence of cerebral, cardiac or peripheral vascular complications. PWV was evaluated after 30-minutes bed resting and used as an index of arterial stiffness, and blood pressure (BP) were assessed. At overnight fast, blood sampling were performed: plasma glucose (PG), hemoglobin A1c (HbA1c), serum lipids, creatinine and von Willebrand factor (vWF), a marker of vascular endothelial cell damage. Urinary albumin excretion ratio (AER), and urinary transferrin (Tf) and type IV collagen (Col-IV) were also determined in morning spot urine sample. Creatinine clearance rate (Ccr) was calculated from the Cockcroft-Gault formula. The subjects were categorized in quartiles based on the PWV value. The analysis was carried out on log-transformed AER, Tf and Col-IV values due to the skew distribution of these variables.
Results: The values of PWV in the first, second, third and fourth quartiles were 7.9+/−0.4 (n=20), 9.2+/−0.4 (n=49), 10.5+/−0.3(n=44) and 12.1+/−0.4 m/sec (n=15), respectively. Systolic BP in the fourth quartile was significantly higher than that in the first quartile, but diastolic BP was not different. No significant differences were observed in PG, HbA1c and serum lipids among four quartiles. Ccr levels in the third and the fourth quartile of PWV were significantly lower than those in the first quartile. Furthermore, vWF, log AER and log Tf were significantly increased through quartiles of PWV. Moreover, log Col-IV levels in the fourth quartile were significantly higher than those in the first quartile. In lineal regression analysis,

age, duration of diabetes, systolic BP, PG, log AER, log Tf and log Col-IV in all subjects were positively correlated, and Ccr was inversely correlated with PWV. Stepwise multivariate analysis in all subjects demonstrated that independent predictive values for PWV were log AER (F=10.92, P=0.001), systolic BP (F=7.96, P=0.006), age (F=7.58, P=0.006) and Ccr (F=5.10, P=0.026), respectively.
Conclusion: These results suggest that AER, systolic BP, age and Ccr are diagnostic predictors of aortic stiffness, and neither urinary Tf nor Col-IV are predictive values of aortic stiffness in elderly type 2 diabetes without macroangiopathy.

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Cardiovascular risk factors and intima-media thickness of the common carotid artery in patients with latent autoimmune diabetes of the adult (LADA)

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Background and Aims: patients with LADA show different anthropometric and metabolic characteristics as compared to the "common" form of type 2 diabetes (T2DM). The aim of this study was to investigate multiple cardiovascular risk factors and the intima-media thickness of the common carotid artery (CCA-IMT), an early marker of atherosclerosis, in LADA (n=26, M/F 13/13) and T2DM (n=23, M/F 15/8) patients.
Materials and Methods: the LADA group included patients who had seropositivity for antibodies to glutamic acid decarboxylase (GAD, RIA method), adult age at diagnosis (>25 yrs old), and at least six months without insulin treatment post-diagnosis.
Results: LADA and T2DM subjects were not different for age (43.8±2.9 yrs for LADA vs. 49.3±2.2 yrs for T2DM, p=0.14), duration of disease (5.48±1.0 yrs for LADA vs. 8.05±1.80 yrs for T2DM, p=0.23), and HbA1c levels (8.42±õ0.40% for LADA vs. 8.19±0.44% for T2DM, p=0.70). However, as compared to T2DM subjects, patients with LADA showed reduced fasting C-peptide levels (1.38±0.30 vs. 3.37±0.39 ng/ml, p=0.0003), a trend toward lower BMI (24.42±0.70 kg/m^2 vs. 25.83±0.94 kg/m^2, p=0.22) and waist (88.2±2.3 cm vs. 92.3±2.2 cm, p=0.17) values, and significantly lower arterial blood pressure levels (systolic blood pressure 120.8±2.8 vs. 130.5±2.8 mmHg, p=0.02; diastolic blood pressure 76.60±1.4 mmHg vs. 81.14±1.7 mmHg, p=0.046). The lipid profile also significantly differed in LADA patients since they had lower total cholesterol (171.7±6.1 mg/dL vs. 191.6±6.7 mg/dL, p=0.033), LDL cholesterol (108.8±5.7 mg/dL vs. 127.2± 6.3 mg/dL, p=0.036), and triglyceride (72.5±7.5 mg/dL vs. 129.3±18 mg/dL, p=0.0061) levels, and higher HDL cholesterol (48.4±2.3 mg/dL vs. 40.5±2.2 mg/dL, p=0.016) levels compared to T2DM subjects. Interestingly, the CCA-IMT, evaluated by high-resolution B-mode ultrasonography, was significantly lower in LADA than in T2DM patients (0.870±0.032 mm vs. 0.955±0.027 mm, p<0.05).
Conclusion: therefore, as compared to T2DM individuals, LADA patients show more beneficial anthropometric and metabolic characteristics and lower cardiovascular risk factors and markers that could be associated with lower atherogenesis and frequency of cardiovascular disease in this diabetes subgroup.

1210

ACE inhibitor/angiotensin II receptor blocker ameliorates secular change in brachial-ankle pulse wave velocity in type 2 diabetic patients

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Background and Aims: Pulse wave velocity (PWV) reflects aortic stifness, being an independent cardiovascular risk predictor in hypertensive and end stage renal disease patients. It is reported that ACE inhibitor/angiotensin II receptor blocker (ACEI/ARB) reduces PWV in essential hypertensive patients. In type 2 diabetic patients, it is well known that PWV is higher than in normal population, but whether intervention of ACEI/ARB improves PWV is obscure. We examined how much secular change of PWV is influenced by chronic administration of ACEI/ARB in type 2 diabetes mellitus patients.
Materials and Methods: We measured brachial-ankle PWV (baPWV) and ankle-brachial index (ABI) by form PWV/ABI (Colin MT Co. Ltd.) in 109 of diabetic in-hospital patients or outpatients in our hospital (whose age ranged 48 to 73 years, 59 male and 50 female, HbA1c; 8.3± 2.3%). We measured baPWV at the beginning and the end of observation period (29±11 months after), and the change in baPWV values during the observation period was referred as ΔPWV. All the subjects were divided into ACEI/ARB treated group (group A, n=41) and non-treated

group (group C, n=68). We investigated clinical profiles and medications such as sulfonylureas, insulin, biguanide, α-GI, pioglitazone, statins, fibrates, anti-platelets and Ca channel antagonists, as well as ACEI/ARB. Those who had low value of ABI (<0.9) were excluded from this study.
Results: Characteristics of the subjects at the beginning and at the end of the observation were as follows; blood pressure (mmHg): 138±18/80±11, 139±21/80±13, baPWV (cm/s): 1730±361, 1772±338, pulse pressure (mmHg): 57±10, 59±11. ΔPWV was 47±286 cm/s. In group A, profiles were as follows; blood pressure (mmHg): 143±19/84±13, 144±19/82±12, baPWV (cm/s): 1829±443, 1758±298, while in group C, blood pressure (mmHg):134±17/78±9, 135±22/77±13, baPWV (cm/s): 1668±298, 1780±359. ΔPWV was significantly ameliorated in group A (group A/group C: −53.7±309/110.6±260, P=0.0049), although values of systolic blood pressure (group A/group C: 0.829±20.7/1.833±18.8) and pulse pressure (group A/group C: 2.355±10.5/1.365±11.0) were not significantly altered during the period. Multiple regression analysis revealed that only administration of ACEI/ARB was associated with ΔPWV (β=−0.303, P=0.0046) in all those drugs.
Conclusion: In the type 2 diabetic patients, chronic administration of ACEI/ARB ameliorates the secular change in baPWV independent of blood pressure.

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The effect of atorvastatin therapy on the elastic properties of arteries in subjects with type 2 diabetes and dyslipidaemia

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Background and Aims: Dyslipidaemia is a major risk factor for cardiovascular disease (CVD) in patients with type 2 diabetes (T2DM), associated with endothelial dysfunction and increased arterial stiffness. Statins are accepted as first-line treatment for such patients, improving endothelial function and exerting a direct antiatherogenic effect in the arterial wall. Pulse wave velocity (PVW) of the aorta, which reflects its elastic properties, has emerged as an important predictor of cardioavascular events. The aim of the study was to assess the effect of atorvastatin on aortic and brachial arterial stiffness by measuring of PWV.
Materials and Methods: A total of 23 patients (7 men and 16 women) with T2DM, mean age 61.6±7.6 years, duration of diabetes 6.8±5.6 years, and dyslipidaemia were examined before, 3, and 6 months after initiation of statin therapy (atorvastatin 10 mg daily). Subjects were eligible if they were 40–75 years of age, had LDL cholesterol (LDL-C) ≥100 mg/dl, and if they did not have clinically apparent macrovascular disease. HBA1c, plasma lipids were determined and carotid-femoral PWV (PWVcf) and carotid-radial PWV (PWVcr) were measured by sequential applanation tonometry (SphygmoCor Vx, AtCor Medical, Sydney, Australia).
Results LDL-C values declined significantly at 3 and 6 months by 34% and 33%, respectively (both P values<0.001). The same was valid for total triglycerides (decline by 33% and 20%, respectively, P<0.04). No significant changes were found in HBA1c and HDL cholesterol levels. PWVcr values declined by 11% at 3 months (P=0.001) and 17% at 6 months (P=0.001). The same was valid for PWVcf at 3 and 6 months (decline by 13%, P=0.006, and by 10%, P=0.004, respectively).
Conclusion: Statin treatment, except of reducing LDL-C and triglyceride levels in patients with T2DM, improved the elastic properties of the aorta and brachial artery after 6 months therapy.

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Atorvastatin improves arterial stiffness in normotensive normolipidaemic persons with type 2 diabetes

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Background and Aims: Increased arterial stiffness is an independent predictor of cardiovascular disease and mortality in middle-aged and older adults.
Materials and Methods: Arterial stiffness was measured by pulse wave velocity (PWV) in brachial-ankle segments by automated oscillometry in 71 normotensive and normolipidaemic subjects with type 2 diabetes (40 males and 31 females).
Results: There was no significant correlation between the mean brachial-ankle pulse wave velocity (baPWV) and duration of diabetes, BMI, waist circumference, waist-hip ratio, glycated hemoglobin, LDL and/or HDL cholesterol or spot urine albumin-creatinine ratio at the baseline. However correlation was found between baPWV and systolic blood pressure. 57 patients showing abnormal baPWV were randomized into 2 groups. Patients in group A (n=29) were given 10 mg atorvastatin daily for 6 months. 28 patients in group B, who served as matched controls, were given placebo for the same duration in a double blind manner with both the investigator and the patient being blinded as to the nature of the tablet being administered (atorvastatin or placebo); baPWV was reassessed after 6 months in all patients.

Conclusion: The atorvastatin group showed statistically significant reduction in baPWV (1712.03±349.9 cm./sec. vs. 1658.81±303.26 cm./sec., p<0.05). Although the placebo group also showed some improvement in mean baPWV (1692.03±425.15 cm./sec. vs. 1672.78±425.11 at baseline, the difference was not statistically significant (p>0.1).

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Aortic vascular smooth muscle cells from insulin-resistant obese Zucker rats show increased proliferative response to serum in comparison to those from lean Zucker rats: involvement of MAPK and VASP activation

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Background and Aims: Insulin resistance is a risk factor not only for cardiovascular atherothrombotic events but also for restenosis after percutaneous revascularization. Vascular smooth muscle cell (VSMC) proliferation, which is involved both in atherosclerosis and in post-angioplasty restenosis, is regulated by a complex sequence of intracellular signaling pathways, which involves activation of Mitogen-Activated Protein Kinase (MAPK) and PKC-induced phosphorylation at Ser 157 of Vasodilator-Stimulated Phosphoprotein (VASP). To clarify whether VSMCs show an increased proliferation attitude in the insulin-resistant states, we aimed to evaluate whether cultured aortic VSMCs from insulin-resistant obese Zucker fa/fa rats -a classical model of insulin resistance- show differences in serum-induced proliferation, MAPK pathway activation and PKC-dependent pSer157 VASP expression in comparison to VSMC from insulin-sensitive lean Zucker fa/+rats.
Materials and Methods: Cultured aortic VSMCs from insulin-resistant obese Zucker fa/fa rats and from insulin-sensitive lean Zucker fa/+rats obtained in primary culture in our laboratory were exposed to different concentrations of foetal calf serum (FCS, 1–10%) for 24–96 hours; at different times, VSMC number, VSMC incorporation of 3H-thimidine, expression of phosphorylated ERK 1 and ERK 2 - steps of the MAPK cascade- and expression of pSer157VASP (Western blot) were measured (n=4, results expressed as mean±SEM and referred to 0% FCS).
Results: In response to FCS exposure at different concentrations and times, VSMCs from obese Zucker rats exhibited a significantly greater proliferation in comparison to VSMCs from lean Zucker rats. For instance, a 24-hour VSMC incubation with 5% FCS induced an increase in VSMC number of 366.6±26.9% in obese vs 166.7±13.3% in lean Zucker rats (p=0.0001) and an increase in 3H-thimidine incorporation of 1380.0±148.54% in obese vs 878.83±46.09% in lean Zucker rats (p=0.009). Incubation (30 min-24 hours) with 1–10% FCS serum significantly increased expression of phosphorylated ERK-1, ERK-2 and pSer157VASP both in VSMCs from obese (p=0.03–0.0001) and in those from lean (p=0.05–0.0001) Zucker rats: the extent of phosphorylation, however, was greater in obese vs lean Zucker rats, as far as ERK-1 (p=0.003–0–0001), ERK-2 (p=0.002–0.0001), and pSer157 VASP (p=0.01–0.0001) is concerned. The serum-induced phosphorylation of VASP at Ser157 was blunted by the PKC-inhibitor staurosporine (1μmol/l) both in obese and in lean Zucker rats. In the same cells, no difference was observed in the proliferative response to insulin.
Conclusions: VSMC from insulin-resistant obese fa/fa Zucker rats showed -in comparison with VSMC from insulin-sensitive lean fa/+rats- increased FCS-induced DNA synthesis and cell proliferation. These effects correlated with an increased activation of the MAPK pathway and with an increased PKC-induced pSer157VASP phosphorylation. The increased proliferative attitude of VSMC in a classical model of insulin resistance provides a further piece of information on the mechanisms involved in the increased risk of cardiovascular atherothrombotic events and of restensosis after angioplasty in the insulin-resistant states.
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Peripheral arterial disease

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Reduced TIMP3 levels in atherosclerotic plaques from patients affected by type 2 diabetes and impaired glucose tolerance

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Background: Tissue Inhibitor of Metalloproteinase 3 (Timp3) is an inhibitor of metalloproteinases (MMPs) and TNF-alpha converting enzyme (TACE) known to be a negative regulator of inflammation, excessive angiogenesis and cell motility. We reported that loss of Timp3 converts the phenotype of Insulin Receptor haploinsufficient mice from normal glucose tolerance to diabetes and vascular inflammation, through up-regulation of TNF-alpha signaling. Furthermore, increased expression of membrane

TNF-alpha in vascular tissues reduced the extent of atherosclerotic plaque growth in a murine model of atherosclerosis. Increased Tace activity has been recently demonstrated to cause angiotensin-II chronic kidney disease. The aim of this study is to investigate alteration in Timp3 expression in tissues from diabetic and non diabetic patiets.
Methods and materials: Here, we explore the levels of Timp3 and TACE by Real Time PCR in: a) skeletal muscle (n=70) and subcutaneous fat (n=106) from patients with obesity (BMI>35) obtained through bariatric surgery, and b) carotid atherosclerotic plaques (n=74) from patients with different degrees of glucose tolerance (NGT, n=41; IGT, n=17; DM2=16 all matched for age, sex, BMI and medications other than hypoglycaemic agents).
Results: In patients with severe obesity divided according to glucose tolerance states (NGT, IGT/IFG, DM2) Timp3 and TACE levels were similar in muscle and fat. By contrast, Timp3 levels (but not TACE) were decreased in atherosclerotic plaques from both IGT and DM2 patients compared with NGT ($P<0.02$ and $p<0.001$, respectively, 1-way ANOVA) (Figure 1). Timp3 levels were inversely correlated to HbA1c ($r=-0.31$, $p<0.019$), LDL Cholesterol ($r=-0.29$, $p<0.029$), Total Cholesterol ($r=-0.31$, $p<0.016$) and C reactive Protein ($r=-0.28$, $p<0.030$). Immunohistochemical analysis confirmed reduced Timp3 levels in plaques from IGT/DM2 patients (n=16) compared with NGT patients (N=16) ($p<0.01$, t test).
Conclusions: Our results suggest that reduced Timp3 expression is a feature of macrovascular disease linked to insulin resistance and type 2 diabetes.

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Carotid atherosclerosis is influenced by visceral adiposity rather than general obesity in type 2 diabetic patients

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Background and Aims: Diabetes mellitus is a major independent risk factor for atherosclerosis. Carotid intima-media thickness (IMT) is a measure of subclinical atherosclerosis and is predictive of future myocardial infarction and stroke. General obesity as indicated by high body mass index (BMI) has been related to increased risk of clinical events of atherosclerotic diseases. Interestingly, there is some evidence that abdominal obesity, as indicated by high waist-to-hip circumference ratio (WHR) or high waist circumference, is even a stronger predictor of atherosclerotic diseases than general obesity. We studied whether obesity, especially abdominal obesity is associated with carotid atherosclerosis in type 2 diabetic patients.
Materials and Methods: One hundred and seventy-nine Korean type 2 diabetic patients (M: 92, F: 87; mean body mass index (BMI): 23.6 kg/m^2; age range: 34–83 years) were recruited. All subjects underwent assessment for the degree and distribution of obesity, the presence of coronary artery disease risk factors, and the presence of diabetic complications, and examined the body composition and body-fat percent by measuring bioelectrical impedance using a body fat analyzer. The carotid IMT was assessed using B-mode carotid ultrasound by measuring generally used parameters, including maximum and mean IMT.
Results: Diabetic patients having visceral obesity (waist to hip ratio > 0.9) showed greater maximum (1.17±0.26 vs. 0.98±0.23 mm; $p=0.001$) and mean (0.84±0.17 vs. 0.70±0.11 mm; $p<0.001$) IMT of the carotid artery than patients without visceral obesity. But there was no significant difference in maximum and mean carotid IMT between patients without general obesity (BMI<23 kg/m^2) and patients with general obesity (BMI ≥ 23 kg/m^2). The correlations between visceral obesity and carotid IMT were higher in women ($r=0.35$) than men ($r=0.28$). Age, gender, duration of diabetes, blood pressure, total-cholesterol concentration, glycated hemoglobin, cardiovascular risk factors and diabetic microvascular complications were not different according to visceral obesity. On univariate analysis, visceral but not general obesity was the major correlate with maximum and mean carotid IMT in both men and women. On multivariate analysis, carotid IMT was an independent determinant of carotid IMT after adjustment for age, gender and smoking. The association of visceral obesity with carotid IMT appeared stronger in women than in men, which however was not confirmed on multivariate analysis.
Conclusion: This study suggested that abdominal obesity rather than general obesity was associated with carotid atherosclerosis in type 2 diabetic patients.

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Non-traditional cardiovascular risk factors as contributors to peripheral arterial disease in type 2 diabetes

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Background and Aim: Screening for peripheral artery disease (PAD) is not common practice in diabetic patients, so that its prevalence is not yet well documented. We evaluated

the PAD prevalence in type 2 diabetic patients, its association with traditional and non-traditional risk factors for atherosclerosis and risk of coronary heart disease (CHD).

Patients and Methods: A cross-sectional study was conducted in 1610 Type 2 diabetic patients (age 61,6±7.0 years; 42% women and 58% men). Ankle-brachial pressure index was measured and PAD was defined as ABPI<0,9. HbA_{1c}, total-, LDL- and HDL-cholesterol (HDL-C), uric acid, fibrinogen, albumin/creatinine ratio were measured and glomerular filtration rate (GFR) estimated by MDRD. CHD risk was calculated by UKPDS Risk Engine.

Results: Overall PAD prevalence was 18% (men: 18%; women: 16%, X^2: p=ns), but increased with age (<40 years: 2%; >70 years: 45,5%; Chi-Square: p<0,0001) and with diabetes duration (<10 years: 42%; >10 years: 58%; Chi-Square: p<0,0001). The prevalence of traditional cardiovascular risk factors such as smoking (24% vs. 21%), hypertension (72,9% vs. 66,5%), obesity (19% vs. 17%), LDL-C>130 mg/dl (18,4% vs. 18,6%), low HDL-C (19% vs. 18%) and high triglycerides (19,7% vs. 17,7%) was not different between patients with and without PAD. PAD patients had higher levels of fibrinogen (370±79 mg/dl vs. 347±76 mg/dl; ANOVA: p<0,0001), higher prevalence of microalbuminuria (21,3% vs. 13,7%; Chi-Square: p<0,05) and lower renal function (GFR 80,7±24 vs. 89,9±22 ml/min/1,73 m^2; ANOVA: p<0,001) as compared to subjects without PAD. In age- and gender-adjusted analysis, ATPIII metabolic syndrome components and traditional cardiovascular risk factors were not associated with PAD, with the exception of current smoking (OR 1,5; CI: 1,07–2,2; p<0,05). In contrast, there was a significant correlation between PAD and poor glucose control (OR 1,45; CI: 1,07–2,08; p<0,05), high pulse pressure (OR 2; CI: 1,46–2,75; p<0,001), reduced GFR (OR 2,16; CI: 1,4–3,3; p<0,0001), microalbuminuria (OR 1,62; CI: 1,11–2,36; p=0,001), fibrinogen (OR 1,78; CI: 1,29–2,47; p<0,001) and uric acid (OR 1,5; CI: 1,03–2,19; p<0,05). Among patients without history of CV disease, 20% of subjects with high CHD risk had ABPI<0,9 while the risk of PAD in patients with high CHD risk (≥20%) was higher than in those with low risk (OR 2,5; CI: 1,47–4,2; p<0,001).

Conclusions: The prevalence of PAD, as determined by ABPI, is high in type 2 diabetic patients and is increased by age, diabetes duration, smoking and non-traditional CV risk factors. Patients with PAD also carry greater CHD risk.

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Is it worthwhile screening for peripheral arterial disease in subjects with type 2 diabetes mellitus? Results from a nation-wide screening study

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Awareness of peripheral arterial disease (PAD) is low among physicians, because the recommended test for its detection (ankle-brachial index -ABI-) is time-consuming and is not usually performed in routine clinical practice. However, PAD is a coronary heart disease (CHD) risk equivalent and a tight control of classical cardiovascular risk factors (CCRF) is recommended in this condition.

Objective: To assess in type 2 diabetes mellitus (T2DM) subjects whether those subjects with PAD have a different control of CCRF in relation to subjects without PAD.

Methods: 31 Spanish Diabetes Departments consecutively screened for PAD (ABI test) 1305 subjects with T2DM (age: 65.4±7.7 yr, women: 52.2%, diabetes duration: 13.4±8.9 yr), recording information on control of CCRF and history of CHD and stroke.

Results: 292 subjects showed PAD (ABI<0.9) (23.10%, CI 95%: 20.78-25.42, after excluding 41 subjects with an ABI > 1.30). According to the presence of other previously known cardiovascular disease (CVD) manifestations (CHD or stroke) and the current diagnosis of PAD, subjects were classified into 4 groups: 1) without previously known CVD and no PAD (n=789); 2) without previously known CVD and

PAD (n=194); 3) with previously known CVD and no PAD (n=182), and 4) with previously known CVD and PAD (n=95). The control of the CCRF was (respectively): Non-smokers: 84.5%, 79.3%, 93.3%, 86.1% (2 vs 3 p<0.01); antiplatelet treatment: 36.1%, 40.2%, 80.2%, 79.0% (2 vs 3 and 2 vs 4 p<0.01); blood pressure<130/80 mmHg: 17.9%, 12.9%, 20.3%, 16.0% (NS); LDL cholesterol<100 mg/dl: 43.8%, 35.5%, 59.6%, 64.3%(2 vs 3 and 2 vs 4 p<0.02); HbA1c<7.5%: 50.7%, 45.5%, 47.6%, 52.4% (NS).

Conclusions: Detection of PAD by the ABI identifies a group of subjects with a poorer control of CCRF than subjects with other clinical manifestations of CVD, thereby increasing the value of ABI in the routine care of subjects with T2DM.

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Concordance of ankle-brachial index and toe-brachial index in patients with diabetes

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Background and Aims: To evaluate the influence of peripheral neuropathy, autonomic neuropathy and arterial wall calcification on the concordance of two screening methods, ankle-brachial index (ABI) and toe-brachial index (TBI), used to assess lower limb perfusion in patients with diabetes.

Materials and Methods: We evaluated 221 diabetic patients (73.8% type 2; 66.6% male) with a mean age of 61.1 ±11.9 years and mean duration of diabetes of 16±11.9 years. Both brachial systolic pressures, and ankle (posterior tibial and dorsalis pedis) and toe systolic pressures were measured using a Doppler probe (8Mhz). For each patient, the lowest of the four ABI and the lowest of the two TBI obtained were used for the analysis. ABI values<0.9 and TBI values<0.5 were considered indicators of peripheral artery disease. Peripheral neuropathy was assessed using Boulton's clinical scale, a 10-g monofilament and a 128 Hz tuning fork. Continuous flow in the waveform doppler display was considered indicator of autonomic neuropathy. An ABI value ≥ 1.30 was considered indicator of arterial wall calcification. Statistical analysis was performed using SPSS software.

Results: The mean difference between ABI and TBI in our population was 0.33±0.25. Patients with arterial wall calcification had a higher mean difference between ABI and TBI than patients without arterial wall calcification (0.87±0.39 vs 0.30±0.21; p=0.002). Mean difference between ABI and TBI was similar for patients with and without autonomic neuropathy (0.36±0.26 vs 0.30±0.24; p=0.057) and for patients with and without peripheral neuropathy (0.34±0.28 vs 0.31±0.21; p=0.423). Among all patients there was a significant linear association (r=0,506; p=0,000) between ABI and TBI. For patients with arterial wall calcification there was no correlation between ABI and TBI (r=−0.070; p=0,857). Autonomic neuropathy and peripheral neuropathy did not modify the relation between ABI and TBI (r=0.562 and 0.492 respectively).

Conclusion: Our findings indicate that in patients with diabetes TBI is the method of choice to evaluate lower limb perfusion in the presence of overt arterial wall calcification as evidenced by an ABI value ≥ 1.30. However, in diabetic patients with autonomic neuropathy or peripheral neuropathy there is a good relationship between ABI and TBI.

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Clinical significance of toe-brachial pressure index in patients with type 2 diabetes

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Background: Peripheral obstructive arterial disease (POAD) is a potent predictor of cardiovascular disease (CVD) in diabetic patients. Medical and/or life style intervention in the earlier stage of POAD may also reduce the risk of CVD mortality of the patients. Although the efficacy of ankle brachial pressure index (ABI) for diagnosing of POAD is well demonstrated, its sensitivity is not so high enough for detecting the early stage of POAD, particularly in type 2 diabetes. More sensitive clinical marker for POAD should be established.

Aims: To assess the efficacy of measurement of toe-brachial pressure index (TBI) using digital cuff in patients with type 2 diabetes.

Materials and Methods: For 155 patients with type 2 diabetes and without apparent clinical symptoms of the peripheral arterial obstruction, ABI and TBI were screened simultaneously. Also, carotid intima-media thickness (IMT) and pulse wave velocity (PWV) were measured in these patients for evaluating the degree of their atherosclerosis. Comparative analyses of both ABI and TBI in relation to other clinical indication of diabetic complications were performed.

Results: Significant positive correlation was found between ABI and TBI. In that relationship, the cut off value of 0.9 in ABI for POAD was equivalent to the value of 0.6 in TBI. In 25 cases whose TBI was under 0.6, 72% of the patients had a clinical history of cardiovascular disease, and this rate was much higher than that of the patients whose TBI was

above 0.6. This group with low TBI had longer duration of diabetes than the group with high TBI. The frequencies of the patients with hypertension and/or hyperlipidemia were also higher in the low TBI group than in the high TBI group. Although 89% of the patients showed low TBI with low ABI, or high TBI with high ABI, the discrepancy was found in 15 patients whose ABI was above 0.9, and their TBI was below 0.6. In these cases with high ABI and low TBI, the average of IMT was 1.42 mm (normal range <1.10 mm), and their average of PWV was 2,200 cm/sec (normal range<1,600 cm/sec).
Conclusion: In this study, TBI is shown to be as effective marker as ABI for detecting POAD with type 2 diabetes. Furthermore, low TBI may reflect the earlier stage of systemic atherosclerosis in some cases with type 2 diabetes than ABI.

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Paradoxical blood flow responses in postmenopausal women with diabetic neuropathy

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Background and Aims: Diabetic neuropathy (DN) is a common cause of morbidity and death among patients with diabetes mellitus (DM). Reduction of nerve blood flow and endoneural hypoxia are attributed to oxidative stress and to alterations in vasoactive agents. Neuropathy alone can be responsible for abnormal blood flow responses in DM. In addition a low blood estrogen level can deteriorate microvascular complications in postmenopausal women with DM. The aim of this investigation was to study a microvascular disorders and effect of estrogen-progestin replacement therapy (EPRT) on microvascular responses in postmenopausal women with DN.
Materials and Methods: We exanimate 55 postmenopausal women with DM (24/31 patients with 1/2 types of DM) and climacteric vasomotor symptoms (mean [±SD] age 53.2± 1.72 years). Written informed consent was obtained from all patients and the institutional review board approved the study. Diagnose of DN was determined by careful neurological examination, electroneurophysiology studies. The wrist and foot digital skin blood flow and microvessels responses to functional tests were assessed by Doppler highfrequency ultrasound examination. We studied the microvessels endothelial function on measuring reactive hyperemia perfusion changes.We investigated the skin vessels response in another extremity to a local cold stimulus. Hemostasis values, glycosylated hemoglobin, serum lipids, plasma levels of total non-enzymatic antioxidants and malondialdehyde were measured. Control group consists from 15 healthy postmenopausal women of the same age. 51 diabetic postmenopausal women received EPRT by estradiol valerate and dienogest during 12 months.
Results: On baseline the paradoxical decreasing of wrist blood flow was obtained in 42% women with DM type 1 and 52% women with DM type 2. All patients with DM type 1 and 71% with DM type 2 showed the paradoxical increasing of skin wrist blood flow to 87% as compared whit basal level on a local cooling. However basal foot digital skin blood flow was significantly reduced and there were no vessel responses to the functional testing. Diabetic women had higher baseline levels of malondialdehyde and lower levels of total non-enzymatic antioxidants as compared with control groups. There were higher body-mass index, higher levels of total cholesterol, LDL-cholesterol and fasting triglycerides in postmenopausal women with DM type 2 as compared with patients with DM type 1 and control groups. Paradoxical vasoconstrictor blood flow responses to reactive hyperemia testing was associated with obesity, high levels of total cholesterol, LDL-cholesterol and malondialdehyde. The poorer nerve functional and antioxidant control determined the abnormal increasing of microvascular blood flow to the cold testing. EPRT restored the flow-mediated microvessels dilatation in 77/67% patients and the vessels decreasing on the cold testing in 50/56% patients. There were decreasing of the total cholesterol, LDL-cholesterol and malondialdehyde due to the EPRT that determined the improvement of nerve function.
Conclusion: The nerve dysfunction in postmenopausal women with DN associated with high total cholesterol and LDL-cholesterol levels, antioxidant control injury and microvessels disorders. EPRT improve quality of life in postmenopausal women with DN by relieved vasomotor climacteric symptoms. In the same time EPRT may have the beneficial influents on peripheral nerve function.

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Systemic trail delivery shows anti-atherosclerotic activity in diabetic apolipoprotein E-Null (ApoE) mice

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Background and Aims: Diabetes mellitus is associated with a marked increase in atherosclerosis. However, the mechanisms by which diabetes promotes vessel disease have not been fully delineated. Previous *in vitro* studies have suggested that TNF-related apoptosis inducing ligand (TRAIL) might be involved in the regulation of vascular biology, but its role in vascular physiopathology is still incompletely understood.
Materials and Methods: Both recombinant human TRAIL and an adeno-associated virus (AAV) vector expressing human TRAIL were used to deliver TRAIL in 6-week-old ApoE-null mice, in which diabetes mellitus was induced by injection of streptozotocin (50 mg/kg daily for 5 days) for 3 months. Untreated diabetic and non-diabetic ApoE-null mice were used as controls. Body weight, glycated haemoglobin, atherosclerotic lesion area of the aorta, aortic atherosclerotic plaque collagen content, cellular proliferation and smooth muscle cell and macrophage infiltration were evaluated.
Results: Diabetes in ApoE-null mice was associated with a significant increase in aortic atherosclerotic plaque area (diabetics: 8.8±1.1% vs controls: 1.4±0.9%; $p<0.001$) and complexity as assessed by a marked increase in plaque collagen content (diabetics: 34±9% vs controls: 17±8%; $p<0.001$), cellular proliferation (diabetics: 16±9% vs controls: 4±2%; $p<0.001$) and macrophage- and alfa-smooth muscle actin-positive cell infiltration (diabetics: 13±7% vs controls: 7±4%; $p<0.001$). Repeated intraperitoneal injections of recombinant human TRAIL and a single intravenous injection of AAV-hTRAIL significantly attenuated the development of atherosclerotic plaques (4.5±0.5% and 5.2±0.7% respectively). Moreover, systemic TRAIL administration also markedly decrease overall cellular proliferation (3.1±2.6% and 2.7±2.3% respectively) and collagen content (19±11% and 20±6% respectively) in the atherosclerotic plaques of ApoE-null animals without significantly affecting the smooth muscle cell content (21±9% and 17±7% respectively), resulting in a reduced severity of the atherosclerotic lesions.
Conclusion: Overall, our data indicate that diabetes-associated atherosclerosis is ameliorated by TRAIL administration and indicate that AAV vector offer a novel method of using TRAIL as an innovative drug for the therapy of diabetic vascular diseases.

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Treatment of diabetic peripheral arterial disease with neuromodulation FREMS

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Background: Peripheral arterial disease (PAD) may be asymptomatic, may be associated with intermittent claudication or may be associated with critical limb ischaemia. Patients with critical limb ischemia should be revascularized to minimize the chance of limb loss.
In absence of tissue damage, also with low ankle arterial pressure and low oxymetric values, there are no consistent evidence to support specific treatment. Presence of peripheral neuropathy, with loss of pain sensation, leads diabetic patients with PAD at high risk of limb loss.
FREMS neuromodulation (frequency rhythmic electrical modulation system), utilized for the treatment of diabetic neuropathy, was demonstrate to produce vasoactive products, in particular VEGF. For these evidences we can suppose that FREMS therapy can be useful in the treatment of PAD when oxymetric values are low.
Aim of the study: This prospective study was designed to determine if the use of FREMS neuromodulation in diabetic patients with peripheral limb ischaemia, is effective on oxymetric values and pain free walking distance improvement.
Materials and methods: Fourteen patients with PAD, were evaluated with transcutaneous oxymetry and with a standardized treadmill testing. 18 limbs with oxymetric values (TcpO2) below 50 mmHg were founded. For each patients oxymetric test was repeated after one month to verify values stability. All patients received ten consecutive session of FREMS therapy. After one and three months we performed a transcutaneous oxymetry and patients with claudicatio (n.6) repeated treadmill walking test.
Results: 18 legs were examinated: baseline oxymetric values were 26.9±14.4 mmHg (mean±SD). After one month, from the end of FREMS therapy, oxymetric values increased to 38.8±20.7 mmHg, with a p value of 0.01 compared to the baseline values. Moreover at 3 months values further increased at 48±10.1, $p<0.01$ compared to baseline values. After FREMS teraphy patients with claudicatio demonstrated improvement in pain free walking distance at one and three months ($p<0.001$).
Conclusions: In diabetic patients with PAD, FREMS therapy seems achieve considerable effects on improvement of oxymetric values and pain free walking distance. In absence of indications for revascularization this therapy can achieve a good amelioration without risks for the patients.

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Endothelial progenitor cells in subjects with pre-diabetes

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Background and Aims: Cumulative cardiovascular risk, and cardiovascular disease status appear to alter number and function of circulating endothelial progenitor cells (EPCs). Furthermore, the level of circulating EPCs seems to predict the occurrence of cardiovascular events. Diabetes has been associated with low levels of circulating EPCs. To investigate whether even subjects with pre-diabetes show altered circulating levels of progenitor cells (CPCs) and endothelial progenitor cells (EPCs).
Materials and Methods: 27 patients with pre-diabetes (8 with impaired fasting glucose, IFG; 14 with impaired glucose tolerance, IGT; and 5 with both IFG and IGT) and 22 age-matched healthy controls were recruited. No subject was on any pharmacological treatment. BMI, waist circumference, OGTT, HbA1c, blood pressure, lipid profile and inflammatory parameters were measured. Metabolic syndrome (MS) was assessed by NCEP/ATPIII criteria. Blood samples were obtained in EDTA tubes in order to assess CPCs (CD34+cells) and EPCs (CD34+KDR+cells) by FACS analysis. Data are expressed as cells number for ml of blood obtained by ISHAGE protocol and as EPCs/CPCs ratio.
Results: No differences were observed in EPCs levels and in EPCs/CPCs ratio in subjects with pre-diabetes compared with controls (EPCs: 532±424 vs 511±348;/ml, p=0.86; EPCs/CPCs ratio: 28.0¢±16.1 vs 22.9±17.2%, p=0.30). Linear regressions didn not show any significant correlation between EPCs level or EPCs/CPCs ratio and clinical (age, BMI, waist, BP) as well laboratory parameters (fasting or 2-h glucose, HbA1c, glucose area under the OGTT curve, lipids, hs-CRP). Within subjects with pre-diabetes, EPCs (516±298 vs 542±494/ml, p=0.88) and EPCs/CPCs ratio (27.6±16.4 vs 28.3±16.4%, p=0.093) were superimposable in those with (n. 11, 40%) and without MS. All controls were free of MS. A positive first-degree family history for type 2 diabetes was present in 29 individuals, 10 controls (45%) and 19 pre-diabetics (70%). EPCs (436±334 vs 651±432/ml, p=0.067) and EPCs/CPCs (21.9±14.3 vs 31.1±18.5%, p=0.069) were marginally lower in subjects with a positive family for diabetes. This trend was present both in controls and in pre-diabetics.
Conclusion: These preliminary results suggest that minor impairments of glucose regulation (pre-diabetes) do not affect the levels of circulating EPCs. Furthermore, in pre-diabetics, levels of EPCs are not modified by the co-occurrence of metabolic syndrome.

PS 110

Erythrocytes, clotting and thrombosis

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Factor IX activation enzyme on erythrocyte membranes causes hypercoagulability in diabetes

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Background and Aims: Macroangiopathy (MA) such as cerebral infarction, myocardial infarction is a common cause of death in diabetes. These complications are thought due to increased intrinsic coagulation activity. We have discovered new enzyme that activate coagulation factor IX (FIX) on erythrocyte membrane in normal and diabetics. FIX activation enzyme may be a serine protease and the enzyme activity may be identical with that present of RBC membranes in another study. These data suggests RBC may play important role in developing angiopathy in diabetes with its blood coagulation activity.
Materials and Methods: Blood was obtained from human adult volunteers including normal (N: n=21) and diabetic patients (DM n=32). Platelet poor plasma (PPP) was prepared by centrifuging the blood. RBCs were washed in HEPES buffer. The enzyme activity on the surface of erythrocyte was estimated from a comparison of fluorescence intensity with a synthetic substrate (Suc(OMe)-Ala-Ala- Pro-Val-MCA). Time course of fluorescence intensity (FI) was measured using a fluorescence spectrophotometer (Hitachi F-1200). Blood coagulation activity was measured by determination of the onset time of coagulation which was monitored using a damped oscillation rheometer (Kawakamil: Biorheology 32:521, 1995). Hypercoagulable blood showed shortened onset time in logarithmic damping factor (LDF) monitored after adding $CaCl_2$ solution to anticoagulated blood sample. Plasma fibrinogen, plasma thrombin, FXa, FXIIa, FXa, FVIIa, tissue factor(TF) was also measured.
Results: The average of glucose levels and HbAlc levels in diabetic patients were 179.0 mg/dL and 7.9%, respectively. The onset time of whole blood coagulation is significantly shorter in DM than in N (24.8 min V.S. 35.7 min).And the onset time of PFP with RBC is also shorter in DM than in N (35.5 min V.S. 46.0 min). Fluorescent observation of FIX activation enzyme is higher in DM than in N (13.4 V.S. 8.3). In diabetics with MA, onset time(Ti) was shorter than in diabetics without MA. And Ti became short by 22% in high PG group. Fluorescent intensity (FI) was higher in diabetics with MA. Plasma TF, thrombin, and fibrinogen levels are higher in DM especially in DM with MA. HbAlc

levels showed significant positive correlation with FI ($p<0.001$) and plasma fibrinogen ($p<0.001$), and showed negative correlation with Ti ($p<0.01$). Plasma TF or thrombin showed no correlation with Ti or FI in diabetics. Plasma FIXa, FXIIa, and FXa showed no difference between N and DM.

Conclusion: FIX plays an important role in blood coagulation because absence or deficiency of its activity results in hemophilia B. However, epidemiological data showed that the plasma FIXc was associated with several CHD risk factors. We revealed that FIX-activation enzyme was located on the RBC membrane from diabetes. Hyperglycemia in diabetes may be a main cause of hypercoagulability that will increase the incidence of MA such as acute coronary syndrome. We observed that Neutrophil elastase can inactivate FIX and this enzyme activity in diabetes. Measurement of FIX activation enzyme on RBC will be an useful marker for hypercoagulability in diabetes.

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Potential reason for diabetic microcirculatory disorders: decreased NO-synthase activity in red blood cells

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Background and Aims: Endothelial dysfunction and diminished bioavailability of nitric oxide (NO) are hallmarks for development of vascular complications in diabetes. Recently, we provided evidence for an active endothelial-type NO-synthase in erythrocytes (RBC-NOS) of healthy volunteers which influences RBC deformability and might also be of importance for regulation of microcirculatory blood flow. We hypothesise that diabetes does not only impair bioavailability of NO by endothelium, but also reduces the NO-pool supplied by erythrocytes.

Materials and Methods: RBC-NOS activity and RBC deformability were determined in healthy and type 2 diabetic subjects (n=10, age-matched). The release of NO was measured after incubation of erythrocytes with L-arginine (3 mM) and inhibition by L-NIO (L-iminoethyl-ornithine hydrchloride, 10 μM). Changes of RBC-NOS activity were assessed by measurement of nitrite in RBC surrounding plasma by gas phase chemiluminescence. The influence of insulin was tested after incubation of erythrocytes with insulin. Phosphorylation of NOS was determined histochemically. RBC deformability was assessed in relation to RBC flow rate through a filter system.

Results: RBC NOS activity was significantly decreased in diabetics (108±43% in healthy volunteers to 46±23% in diabetics). In parallel RBC-NOS dependent deformability of RBC is significantly reduced (10±5% in diabetics and 15±8% in healthy volunteers). In both collectives altered NOS activity and RBC deformability correlate significantly. Insulin significantly increased the release of RBC-NO and activated RBC-NOS by phosphorylation. In erythrocytes of diabetic patients both effect of insulin on RBC-NOS were blunted.

Conclusion: RBC-NOS activity is drastically impaired in type 2 diabetic patients and is resistant to activation by insulin. Both effects may contribute to the reduced deformability of erythrocytes and to the deleterious microcirculatory damages observed in many diabetic patients. This defect may also contribute to the close association of insulin resistance and microvascular defects. Treatment of RBC NOS may therefore be a potential target to prevent an impairment of microcirculatory blood flow and may offer interesting new perspectives in therapy of diabetic patients.

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Tissue plasminogen activator (tPA) activity is an early and independent marker of LEAD in type 2 diabetes

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Background and Aims: Age, hypertension and smoking are well-known risk factors for the development of lower extremity arterial disease (LEAD). In addition, hyperglycemia, dyslipidemia and duration of diabetes are of major importance in diabetic subjects. In this cross-sectional study we addressed the relationship between early, asymptomatic LEAD and "classic" risk factors for LEAD, markers of inflammation, fibrinolysis and adipose-derived factors.

Subjects and Methods: We studied 226 subjects (75 type 1-, 69 with type 2- and 82 non-diabetic, 113 men, 113 women, age 30-70 years) without previously known or suspected LEAD. Peripheral blood pressures were measured at the level of the ankle (ABP) or the great toe (TBP) using a Doppler pen or a pulse oximetry sensor, respectively, for pulse detection. Toe and ankle blood pressure indices (ABI and TBI) were calculated by dividing ABP and TBP by the systemic, brachial, blood pressure. The subjects were divided into two groups; with normal (n=183) and reduced peripheral blood pressure (n=43), respectively. Reduced blood pressure was defined as at least one absolute blood pressure (ABP or TBP) or index (ABI

or TBI) below the normal range: ankle<98 mmHg, toe<79 mmHg or ABI<0.9, TBI<0.74 (cut-offs are means minus 2 SDs for controls in a previous, larger study; Sahli et al, Angiology, 2004). Blood samples were obtained for measurement of lipids, HbA1c, hs-CRP, TNF-alpha, IL-6, adiponectin, leptin, FFA, tPA-activity, tPA-mass, PAI-1 activity and tPA-PAI-1 complex.

Results: In logistic regression analyses, age ($p<0.05$), hypertension ($p<0.001$), diabetes ($p<0.01$) and high levels of HbA1c ($p<0.001$), FFA ($p<0.01$) and also TNF-alpha, IL-6, adiponectin and tPA-activity (all $p<0.05$) were all associated with impaired peripheral circulation (reduced ABP, TBP, ABI or TBI). When adjusting for the "classic" risk factors, i.e. age, hypertension, smoking and HbA1c, only tPA-activity, among inflammatory, adipose-derived or fibrinolytic factors, was an independent risk marker for LEAD ($p<0.05$). When analyzing each group of subjects, i. e. type 1-, type 2- and non-diabetics, separately, tPA-activity remained an independent risk marker only among subjects with type 2 diabetes ($p=0.03$).

Conclusion: In addition to the "classic" risk factors, tPA-activity may be an independent and early marker for LEAD in type 2 diabetes. Thus, an altered fibrinolytic activity could indicate and possibly contribute to atherosclerosis development in the lower extremities.

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Advanced glycation end products stimulate platelet phosphatidylserine externalisation: a role for $5HT_{2A}$ receptors and protein kinase C δ but not caspase-3

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Background and Aims: Levels of advanced glycation end products are increased in individuals with diabetes and/or end stage renal disease (ESRD) and may contribute to the greatly increased cardiovascular risk in these patients. Platelets play a crucial role in early events leading to inflammatory responses at sites of vessel wall injury. Externalisation of the membrane phospholipid, phosphatidylserine (PS), via activation of protein kinase C δ (PKCδ), has been proposed as a key event in aggregation/adhesion of platelets. Alternatively, PS externalisation has also been implicated as a marker for apoptosis when it occurs in a Caspase-3-dependent manner. A specific 'receptor for AGE', RAGE, has been identified, but its presence on platelets has not been demonstrated and it is clear that not all effects of AGE are mediated via RAGE. In particular, a previous study demonstrating additive effects of AGE with ADP showed that they could be blocked via an inhibitor of the $5HT_{2A}$ receptor.

The aim of this study was to investigate our hypothesis that AGE elicit platelet PS externalisation via PKCδ, but independently of Caspase-3, and to identify the AGE receptor(s) involved.

Materials and Methods: Platelet-rich plasma (PRP) was prepared by centrifugation of whole blood from healthy individuals and PS externalisation was quantified by a fluorescence-activated cell-sorter using Annexin V-FITC to determine PS and with CD61-PerCP as a platelet marker. Human serum albumin-AGE (HSA-AGE) was prepared by incubating HSA with 1M glucose under sterile conditions for six weeks followed by extensive dialysis. PKCδ activity was blocked by Rottlerin, Z-DEVD-FMK was used to inhibit Caspase-3 and Ritanserin was employed as a specific $5HT_{2A}$ receptor antagonist.

Results: 0.5 mg/ml HSA-AGE elicited a rapid increase in PS externalisation in a sub-population of human platelets. This response was maximal after 10 minutes and still significant after 30 minutes with an optimal effect at 0.5 mg/ml HSA-AGE (10263±171 vs 2051±164 total fluorescent binding sites, $n=3$; $p<0.001$). 10 minutes preincubation with the PKC inhibitor, rottlerin, at a concentration specific for the PKCδ isoform, (10 μM), totally inhibited PS externalisation in response to 0.5 mg/ml HSA-AGE. Similarly, a 30 minute preincubation with the $5HT_{2A}$-specific receptor antagonist, Ritanserin, also prevented PS externalisation in response to 0.5 mg/ml HSA-AGE in a dose-dependant manner with complete blockade at 1 μM. In contrast, 30 minutes preincubation of PRP with an optimal concentration of the Caspase-3 inhibitor, Z-DEVD-FMK (2 μM), had no effect on HSA-AGE-mediated PS externalisation.

Conclusion: The data demonstrate that HSA-AGE rapidly stimulates platelet PS externalisation via PKCδ in a time- and concentration-dependent manner. Moreover, the observation that this response is independent of Caspase-3 suggests that it is linked to platelet aggregation/adhesion rather than apoptosis. In addition, our novel finding that HSA-AGE-mediated PS externalisation is completely inhibited by Ritanserin suggests that this effect is mediated through $5HT_{2A}$ receptors rather than RAGE. It may contribute to the increased cardiovascular risk observed in individuals with diabetes and/or ESRD.

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Changes in the level of soluble and thrombocyte surface P-selectin and the number of thrombocyte derived microparticles in diabetes mellitus

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Background and Aims: Thrombocyte hyperfunction is well-known in diabetes mellitus but the causal relationship with diabetic angiopathy is still unanswered. Microparticles are small fragments broken off from the formed elements of the blood and are biologically active, can markedly influence thrombus formation and inflammatory processes. The number of thrombocyte and leukocyte microparticles were both increased in Type 2 diabetes mellitus, and there was a relationship between increased thrombocyte microparticle number and nephropathy. Our aims were to determine the activation level and the number of thrombocyte microparticles and to measure thrombocyte activation in the circulation of diabetic patients and healthy controls. In addition, P-selectin T715P polymorphism was also determined. We investigated the relationship between microparticle number, thrombocyte activation, P-selectin polymorphism and the state of carbohydrate metabolism (HbA1c, existent blood sugar level), carotis intima-media thickness and the occurrence of nephropathy.
Materials and Methods: 100 patients with type II (mean age: 54.89±7.81 years, duration of diabetes: 6.89±5.82 years), 40 patients with type I diabetes mellitus (mean age: 34.29±10.08 years, duration of diabetes: 10.00±10.17 years) and 62 healthy controls (mean age: 39.19±6.12 years) were included in the study. CD42a indicated the presence of thrombocyte microparticles, while the concomitant occurrence of CD62 (P-selectin) reflected activation. Thrombocyte activation was also monitored by CD62. Measurements were performed by flow cytometry. Carotis intima-media thickness was determined by ultrasound, while the presence of nephropathy was demonstrated by determining urine albumin levels following a 24-hour urine collection.
Results: The activity and number of thrombocyte microparticles were significantly increased in both diabetic groups compared to the controls [*thrombocyte microparticle number* - type I diabetes: 360±199 MP/μl, type II diabetes: 311±340 MP/μl, control: 122±68.5 MP/μl, type I and type II diabetes vs. control p<0,0001; *thrombocyte microparticle activity (mean fluorescence intensity)*: type I diabetes: 68.2±48.1, type II diabetes: 76.5±102.6 control: 18±8.7, type I and type II diabetes vs. control p<0,0001]. There was a significant positive correlation between thrombocyte microparticle number, P-selectin and soluble P-selectin levels and HbA1c, existent blood sugar level and intima-media thickness. We could not detect any relationship with microalbuminuria.
Conclusion: The level of thrombocyte activation and the number of thrombocyte microparticles are significantly increased in diabetes. Our results suggest that there is a strong correlation between these values and the intima-media thickness, although the causal relationship could not be established. Their role in the pathogenesis of macrovascular complications is likely and their increased level indicate a greater risk for the development of vascular disease. The T715P polymorphism of soluble P-selectin doesn't have any effect on diabetic macrovascular complications.
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Prognostic value of plasma soluble P-selectin and von Willebrand factor as indices of platelet activation and endothelial dysfunction in 234 patients with hypertension with and without diabetes mellitus

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Background and Aims: Abnormal plasma markers of a prothrombotic state have been described in hypertension. We hypothesized that raised plasma levels of soluble P-selectin (sP-sel, an index of platelet activation) and/or von Willebrand factor (vWf, an index of endothelial damage/dysfunction) might predict vascular events in patients with hypertension with or without diabetes mellitus [DM].
Materials and Methods: We measured sP-sel and vWF levels by ELISA in 234 participants (who did not have prior cardiovascular events) and related these indices to subsequent cardiovascular events over a mean follow up period of 59 months.
Results: Plasma sP-sel levels were a significant predictor of myocardial infarction (P=0.03), with the greatest risk for those with the highest levels of sP-sel, but were not predictive of cerebrovascular events (P=0.53), or composite cardiovascular endpoints (P=0.06). No significant relationships were found between vWF levels and outcome. There was no relationship to the presence or absence of DM at baseline or subsequent development of DM during the follow-up period (Table 1). **Conclusion**: Among patients with hypertension, raised levels of sP-sel (platelet activation) were predictive of coronary events. vWf (endothelial

damage/dysfunction) did not predict subsequent cardiovascular events. sP-sel might represent a target for novel therapies or an adjunctive aid to risk stratification in the setting of hypertension. The initial presence of DM or the subsequent development of DM during the follow-up period had no effect on the levels of these prothrombotic markers. Nevertheless, given the high coexistence of hypertension (up to 60%) in patients with DM, the results of this study will be applicable to many patients with DM.

Table 1. Cross-sectional data on levels of von Willebrand factor (vWF), soluble P-selectin (sP-sel) and demographics of 234 treated patients with hypertension (with and without cardiovascular (CV) events) after a mean follow-up period of 59 months.

Characteristics at baseline	Patients with hypertension		
	Patients without CV Events	Patients with CV Events	P value
Age in years±(SD)	63.4±8.1	64.2±6.7	0.55
Number of Patients (%)	183	51	-
Male gender (%)	158 (86.3)	44 (86.3)	0.99
Smoking history (%)	39 (21.3)	17 (33.3)	0.09
Caucasian ethnicity (%)	156 (85.2)	42 (82.4)	0.93
Body-mass index (kg/m^2)±SD	29.0±4.2	29.8±5.0	0.25
Systolic BP (mmHg)±SD	148±15	151±15	0.13
Diastolic BP (mmHg)±SD	84.4±8.8	83.9±7.7	0.68
Diabetes at baseline (%)	43 (23.5)	19 (37.3)	0.07
Diabetes at the end of follow-up (%)	61 (33.3)	23 (45.1)	0.13
Fasting plasma glucose (mmol/l) ±SD	6.11±1.12	6.14±1.37	0.88
Serum cholesterol (mmol/l)±SD	5.44±1.17	5.26±1.14	0.32
Serum triglycerides (mmol/l) [IQR]	1.3 [0.9–1.8]	1.1 [0.8–1.5]	0.05
HDL cholesterol (mmol/l)±SD	1.22±0.35	1.12±0.25	0.04
von Willebrand factor (IU/dl)±SD	134±34	131±33	0.59
soluble P-selectin (ng/ml) [IQR]	61 [50–78]	70 [45–97]	0.23

1230

Platelet soluble glycoprotein V, a new thrombosis marker, is increased in diabetic patients in relation with poor glycaemic control

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Background and Aims: Diabetes is a major risk factor for cardiovascular disease. Soluble GPV (sGPV) is a new marker of thrombosis released from the surface of thrombin-exposed platelets. Diabetic patients with poor glycemic control are at increased risk of developing thrombotic cardiovascular complications. Therefore the aim of this study was to measure plasma levels of sGPV in diabetic patients according to glycemic control.

Materials and Methods: Patients and control subjects were prospectively enrolled between January 2002 and November 2004. Plasma levels of sGPV, thrombin-antithrombin complexes (TAT), platelet factor 4 (PF4) and von Willebrand factor (vWf) were determined in 260 diabetic patients (76 with type 1 and 184 with type 2 diabetes mellitus) and in 175 control subjects.

Results: sGPV was significantly increased in diabetic patients as compared to control subjects (median [interquartile range]: 38.7 [29.6–54.0] vs 29.0 [24.1–35.5] ng/mL; p<0.0001). In the population of diabetic patients, multivariate analysis showed that sGPV levels were strongly associated to glycemic control (hemoglobin A1c: 2.93 [1.50–5.71]; p=0.002; plasma fasting glucose: 2.08 [1.10–3.95]; p=0.025), duration of the disease (2.33 [1.12–4.83]; p=0.023) and platelet count (2.11 [1.25–3.58]; p=0.005). In parallel, TAT, PF4 and vWF levels were increased in diabetic patients independently from hemoglobin A1c and plasma glucose.

Conclusion: sGPV is significantly increased in diabetic patients. There is a tight correlation between sGPV and hyperglycemia. sGPV release could be due to thrombin or to circulating platelet metalloproteases present in diabetes. sGPV is a new tool to study the mechanisms linking hyperglycemia and cardiovascular disease.

1231

A new mechanical compression device improves circulation in critical limb ischaemia - a pilot study

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Objectives: Critical limb ischemia (CLI) is one of the most challenging problems in vascular medicine; treatment failure will most probably result in limb loss. Patients who suffer from CLI (rest pain, non healing ulcers and

gangrene) are usually treated by angioplasty or bypass surgery. The relative low patency rate of the above procedures and the high risk patients (diabetic and cardiac morbidity) limits the success and long-term limb salvage. The current study evaluates the effect of a small home-use and portable mechanical calf compression device in patients that do not qualify for vascular treatment.

Methods: Nine (9) patients with CLI were studied prospectively, 6 of them were diabetic. All patients did not meet the criteria for vascular treatment (based on angiography and co-morbidities). Patients used the device 6 hours daily 5 days a week for 6 weeks and were evaluated at baseline, 3 and 6 weeks with a follow-up visit at 12 weeks. Evaluation included: Common Femoral Artery Flow Volume (AFV) using duplex ultrasound, Ankle Brachial Index (ABI), Transcutaneous Oxygen Pressure ($TcpO_2$) and Tissue Laser Doppler (TLD). A graded pain scale was used for pain assessment at each visit.

Results: Preliminary data demonstrates that $TcpO_2$, AFV and TLD increased significantly in the treated leg and gradually returned to baseline once therapy ended (table). Mean $TcpO_2$ increased up to 10 times the baseline value at 6 weeks following treatment. Resting ABIs did not change during the treatment. The level of pain decreased from 8 to 6.5 following 6 weeks of treatment.

Conclusion: The studied device enhances $TcpO_2$, TLD and AFV when used daily. This may be due to improved venous drainage and decreased peripheral resistance, which led to an increased arterial flow and oxygen supply to the tissue. The effect was gradually reversed after suspension of treatment. The results presented here are preliminary in an on-going study and the effect on limb salvage and wound healing is unclear but encouraging. Further studies are needed to determine the optimal role of this mechanical compression device in the treatment of CLI and potentially diabetic foot patients.

Table: Mean (±standard deviation) of $TcpO_2$, TLD, and AFV at all time points

	Baseline	Treatment period		Follow up
	Day 0	**3 weeks**	**3 months**	**3 months**
$TcpO_2$ [mmHg]	12.7±12.53	33.4±18.98	36.6±20.33	25.5±18.6
TLD [ml/min/ 100 gr tissue]	1.38±0.66	1.76±0.43	1.91±0.43	1.55±0.70
AFV [ml/min]	118.4±33.43	107.6±34.13	115.2±49.41	85.1±10.98

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PS 111

Lipid metabolism and lipid-lowering therapy

1232

High prevalence of low HDL-cholesterol among European type 2 diabetes patients receiving treatment for dyslipidaemia: data from a pan-European survey

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Background and Aims: The objective of the study was to determine the prevalence of low HDL-cholesterol according to current National Cholesterol Education Program and International Diabetes Federation criteria (<1.03 mmol/L [<40 mg/dL] in men and <1.29 mmol/L [<50 mg/dL] in women) in European type 2 diabetes patients receiving treatment for dyslipidaemia.

Material and methods: The pan-European Survey of HDL-cholesterol measured lipid profiles in 8545 patients followed by a specialist for dyslipidaemia in 11 European countries. Within this population, 3866 patients had a diagnosis of type 2 diabetes mellitus (T2DM).

Results: Compared with non-diabetic subjects, the T2DM population were more likely to be overweight or obese, and were more likely to have a history of hypertension or prior myocardial infarction (MI). Most patients with or without T2DM received treatment with lifestyle modification (87% vs. 84%, respectively) and/or pharmacologic lipid-modifying therapy (84% vs. 82%), usually a statin (89% vs. 91%). Similar proportions of patients with or without T2DM received fibrates combined with a statin (2% vs. 1%), fibrate monotherapy (7% vs. 6%) or other treatments (4% vs. 3%). Mean (±SD) HDL-cholesterol (mmol/L) was lower ($p<0.001$) in T2DM (1.22±0.37) than in subjects without T2DM (1.35±0.44). Mean (SD) HDL-cholesterol was also significantly ($p<0.001$) lower in men with T2DM vs. men without T2DM (1.16±0.34 [n=2140] vs. 1.26±0.39 [n=2733]), and in women with T2DM vs. without T2DM (1.32±0.38 [n=1527] vs. 1.53±0.46 [n=1510]). Consistent with these observations, low HDL-cholesterol was more common in T2DM vs. no T2DM for all subjects (45% vs. 30%), men (40% vs. 30%) and women (52% vs. 31%).

The T2DM group (men and women combined) also had higher mean triglycerides (TG) 2.32±2.10 vs. 1.85±1.60 mmol/L in the non-T2DM group ($p<0.001$), but lower mean LDL-cholesterol (3.02±1.05 vs. 3.30±1.14 mmol/L; $p<0.001$) and lower mean total cholesterol (5.12±1.32 vs. 5.38±1.34 mmol/L; $p<0.001$). The T2DM group were

significantly more likely than the no T2DM group (see table) to have high TG, low HDL-cholesterol and high TG, or very low HDL-cholesterol (<0.9 mmol/L).

Conclusions: Low HDL-cholesterol is common among patients treated for dyslipidaemia in Europe, despite widespread use of statins in this population. Overall, half of women and two-fifths of men with T2DM had low HDL-cholesterol, more than half of men and women had high TG, and about one-third of the population had both lipid disorders. Low HDL-cholesterol is an independent risk factor for coronary heart disease, and physicians should be aware of the high risk of low HDL-cholesterol in their statin-treated patients, and adjust therapeutic regimens accordingly.

	T2DM (n=3866)	No T2DM (n=4436)	p
Demographics/disease characteristics			
Males (%)	58	64	<0.001
Mean age (y)	64 (10)	61 (12)	<0.001
Mean BMI (kg/m^2)	31 (6)	28 (4)	<0.001
Mean waist circumference (cm)	104 (15)	97 (14)	<0.001
Mean SBP/DBP (mmHg)	141 (19)/81 (10)	137 (18)/80(10)	<0.001
Mean FBG (mmol/L)	8.3 (2.8)	5.6 (1.4)	<0.001
BMI ≥ 25 kg/m^2 (%)	85	69	<0.001*
BMI ≥ 30 kg/m^2 (%)	48	25	
Hypertension (%)	82	64	<0.001
Prior MI (%)	23	26	0.0015
Lipids			
Low HDL-C (%)	45	30	<0.001
High TG (%)	57	42	<0.001
Low HDL-C and high TG (%)	32	19	<0.001
HDL-C<0.9 mmol/L (%)	18	12	<0.001

*For the overall distribution of BMI. FBG: fasting blood glucose. Figures in parentheses are SD.

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Endothelial dysfunction in type 2 diabetes mellitus is associated with decreased cholesterol absorption and increased cholesterol synthesis

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Background and Aims: Metabolic abnormalities, associated with Type 2 diabetes, feature a novel factor of low cholesterol absorption and high cholesterol synthesis. We hypothesized an association of endothelial dysfunction and disturbances of cholesterol turnover predisposing to an advanced atherosclerosis in patients with type 2 diabetes.

Materials and Methods: 75 patients, 30 with type 2 diabetes and 30 non-diabetic subjects with a history of cardiovascular disease, and 15 healthy subjects were studied. Plasma sterols, soluble adhesion molecules sCD14, sCD40 Ligand, monocyte chemo-attractant protein-1 (MCP-1), E- and P- selectins were measured with and without atorvastatin therapy.

Results: The patients with Type 2 diabetes mellitus showed significantly higher body weight, body mass index, systolic blood pressure, glycaemia and glycosylated haemoglobin. Quicki insulin sensitivity index was significantly lower. The diabetic patients had significantly higher levels of lathosterol, lathosterol/cholesterol, and lower levels of sitosterol and campesterol and the sterol/cholesterol index. Plasma levels of hsCRP, E-selectin and MCP-1 were significantly higher in patients with diabetes than in healthy subjects. The parameter of MCP-1 significantly correlated with lathosterol (p=0,0266) in the cohort of patients treated with statin. The diabetic patients without statin therapy showed a significant correlation of sE-selectin (p=0,0138) with the marker of cholesterol absorption - sitosterol and sitosterol/cholesterol ratio; the relationship disappeared with statin treatment. The diabetic patients without statin therapy showed a significant negative correlation of sP-selectin with the marker of cholesterol synthesis - lathosterol (p=0,0335); the relationship disappeared with statin treatment.

Conclusion: Endothelial dysfunction in Type 2 diabetes mellitus is associated with parameters of decreased cholesterol absorption and increased cholesterol synthesis thus predisposing to adverse cardiovascular outcome of Type 2 diabetes mellitus.

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Increased cholesterol efflux from cultured fibroblasts to plasma from hypertriglyceridaemic type 2 diabetic patients: role of pre β-HDL and lipid transfer proteins

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Background and Aims: Cellular cholesterol efflux represents an early step in the reverse cholesterol transport (RCT) pathway, whereby excess peripheral cholesterol is transported back to the liver. We tested whether, in type 2 diabetes, hypertriglyceridemia is accompanied by increased pre β-HDL and whether such changes would affect the cellular cholesterol efflux-promoting ability of plasma.
Materials and Methods: In 28 hypertriglyceridemic and 56 normotriglyceridemic type 2 diabetic patients, treated with diet alone or in combination with oral glucose lowering agents, and in 56 normotriglyceridemic control subjects, we determined plasma lipids, HDL lipid composition, plasma pre β-HDL and pre β-HDL formation (both expressed in % of plasma apo A-I), cholesteryl ester transfer protein (CETP) concentration, phosphoplipid transfer protein (PLTP) activity and the ability of plasma to stimulate cholesterol efflux out of cultured human fibroblasts.
Results: HDL cholesterol and HDL phospholipids were lower, whereas HDL triglycerides, plasma pre β-HDL and pre β-HDL formation, as well as plasma CETP concentration and PLTP activity were higher in hypertriglyceridemic diabetic patients than in control subjects (all $p<0.05$ to $p<0.01$). Except for lower HDL cholesterol and higher PLTP activity ($p<0.01$), these variables were not different between normotriglyceridemic diabetic patients and control subjects. Cellular cholesterol efflux to plasma from hypertriglyceridemic diabetic patients was increased compared to control plasma (9.0±0.7% per 4 h vs 8.3±0.9% per 4 h, $p<0.01$), but did not differ between plasma from normotriglyceridemic diabetic patients (8.4±0.8% per 4 h) and control subjects. Multiple linear regression analysis demonstrated that, in all subjects combined, cellular cholesterol efflux to plasma was positively determined by pre-β HDL formation, HDL phospholipids, PLTP activity and HDL triglycerides (multiple $r=0.49$), but not by the diabetic state.
Conclusions: Cellular cholesterol efflux to plasma from hypertriglyceridemic diabetic patients is enhanced, probably as a result of increased pre β-HDL, altered HDL lipids and increased PLTP activity. This early step in RCT is unchanged in normotriglyceridemic diabetic patients.
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Efficacy and tolerability of rosuvastatin and atorvastatin when force-titrated in patients with the metabolic syndrome as defined by the IDF: results from the ECLIPSE Study

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Background and Aims: The metabolic syndrome (MeS) is a complex constellation of disorders that increase the risk of coronary heart disease (CHD). Many patients, particularly those at high risk of CHD, fail to reach their LDL-C goals due to suboptimal treatment. The effect of rosuvastatin (RSV) 10–40 mg and atorvastatin (ATV) 10–80 mg over 24 wks was compared in ECLIPSE, an open-label, randomised, multinational, multi-centre, parallel-group study conducted in 118 centres in Canada and Europe.
Materials and Methods: After a 6-wk dietary lead-in period, 1036 patients with LDL-C ≥4.14-<6.47 mmol/L and CHD, a CHD-risk equivalent or clinical evidence of atherosclerosis were randomised to force-titrated treatment with RSV (10, 20 and 40 mg for 6, 6 and 12 wks) or ATV (10, 20, 40 and 80 mg each for 6 wks). In this analysis, we report on 2003 European LDL-C goal achievement and percentage change from baseline in lipid levels in patients with or without the MeS (based on the IDF 2005 definition), and the overall ITT (intention-to-treat) study population. Statistical comparisons were performed on the overall ITT population only.
Results: In the overall ITT population (n=1015), 66.7% of patients (RSV, n=354; ATV, n=323) had the MeS at baseline. Significantly more patients treated with RSV than ATV achieved their 2003 European LDL-C goal at all time points ($p<0.001$, Table), and goal achievement for RSV and ATV was similar in patients with or without the MeS (Table). Throughout the study, in the overall ITT population, changes from baseline in LDL-C ($p<0.001$), TC ($p<0.05$), and HDL-C ($p<0.01$) were significantly greater for RSV than ATV. Lipid changes with RSV and ATV in patients with or without the MeS were consistent with those for the overall ITT population (wk 24 data in Table). Analysis of LDL-C goal achievement and changes in lipid levels was also performed for patients with the MeS as defined by NCEP ATP III, and the results were similar to those presented here. Overall, both treatments were well tolerated.
Conclusions: In the ECLIPSE study, RSV 10–40 mg was more effective than ATV 10–80 mg at enabling patients to achieve their 2003 European LDL-C goals and improving the atherogenic lipid profile. The benefits of RSV treatment were consistent in patients with and without the MeS and in the overall ITT population.

	Overall ITT		With metabolic syndrome		Without metabolic syndrome	
	RSV (n=505)	ATV (n=510)	RSV (n=354)	ATV (n=323)	RSV (n=147)	ATV (n=180)
% patients at LDL-C goal*						
wk 6 (RSV 10 mg, ATV 10 mg)	52.4†	28.8	52.3	28.8	52.8	29.4
wk 12 (RSV 20 mg, ATV 20 mg)	71.3†	45.7	72.5	45.4	67.6	47.7
wk 18 (RSV 40 mg, ATV 40 mg)	80.4†	64.0	80.0	61.9	80.9	68.6
wk 24 (RSV 40 mg, ATV 80 mg)	82.8†	73.3	81.6	71.0	85.3	77.4
% change from baseline at wk 24						
LDL-C	−57.3†	−52.2	−57.3	−52.1	−56.8	−52.5
TC	−41.3‡	−39.5	−41.5	−39.7	−40.4	−39.2
HDL-C	+8.4†	+1.8	+8.7	+2.8	+7.9	+0.6
TG	−24.6	−28.0‡	−25.7	−29.5	−21.4	−25.3

*2003 European LDL-C goal:<2.5 or 3.0 mmol/L, depending on risk category
†p<0.001 vs ATV
‡p<0.05

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Lipid-lowering effects of colesevelam hydrochloride (HCl) in patients with type 2 diabetes

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Background and Aims: The importance of lipid control in patients with type 2 diabetes mellitus (T2DM) has been well established.
Materials and Methods: The lipid-lowering effects of colesevelam HCl in patients with T2DM were evaluated in a prospective, randomized, double-blind, placebo-controlled, parallel-group pilot study. The trial was comprised of a 4-week, placebo run-in period followed by a 12-week active treatment period. Eligibility criteria included a diagnosis of T2DM, HbA1C 7.0%–10.0% inclusive at the time of randomization, serum triglycerides ≤300 mg/dL, BMI ≤40 kg/m^2, and at least 3 months of treatment with a stable dose of a sulfonylurea, metformin or their combination. Eligible subjects were maintained on their existing antidiabetic regimen and were randomized to colesevelam HCl (3.75 g/d) or placebo (6 tablets/d). Sixty-five patients (31 receiving colesevelam HCl and 34 receiving placebo) were randomized.
Results: Colesevelam HCl treatment resulted in significant reductions compared to placebo in LDL-C (−11.7%; *P*=0.007), total-C (−7.3%; *P*=0.019), apolipoprotein B (−11.8%; *P*=0.003), and LDL particle concentration (−209.6 nmol/L; *P*=0.037). Of particular interest is the fact that triglycerides were not significantly increased with colesevelam HCl compared to placebo (treatment effect+8± 14 mg/dL; *P*=0.570). No significant treatment differences for other lipid parameters were noted.
Conclusion: These data indicate that colesevelam HCl is a useful agent for lowering LDL-C, total-C, apolipoprotein B, and LDL particle concentration in patients with T2DM.

	LDL-C (mg/dL)		Total-C (mg/dL)		Apo-B (mg/dL)		LDL Particle Concentration (nmol/L)	
	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12	Baseline	Week 12
Colesevelam (n)	123 (25)	108 (27)	208 (27)	197 (17)	129 (27)	118 (27)	1559 (26)	1428 (26)
Placebo (n)	120 (30)	122 (32)	196 (32)	202 (32)	123 (31)	129 (31)	1524 (29)	1603 (29)

Supported by Sankyo Pharma Inc.

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Colesevelam HCl improves glycaemic control in patients with type 2 diabetes (T2DM): a pilot study

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Background and Aims: Colesevelam HCl, a bile acid sequestrant, is indicated for lowering LDL-cholesterol, but may also affect blood glucose levels.

Materials and Methods: The effect of colesevelam HCl on glycemic control in patients with T2DM, not adequately controlled with their current anti-diabetic regimens, was evaluated in a prospective, randomized, double-blind, placebo-controlled, parallel-group pilot study. The trial consisted of 4-weeks of placebo run-in followed by 12-weeks of active treatment. Eligibility criteria included a T2DM diagnosis, at least 3 months of treatment with a stable dose of a sulfonylurea, metformin or their combination, and A1C 7.0%–10.0% inclusive at randomization. Eligible patients were randomized to colesevelam HCl (3.75 g/d) or placebo (6 tablets/d). Sixty-five patients were randomized; 59 completed the study. Differences between groups were evaluated by Analysis of Covariance, with baseline values as covariates, and are expressed as least square (LS) means±standard error (SE).
Results: Fasting plasma glucose (LS mean±SE) was significantly lower in the colesevelam HCl group compared to the placebo group at weeks 4 (−23±9 mg/dL, *P*=0.016) and 8 (−18±7 mg/dL, *P*=0.011), and tended to be lower at week 12 (−14±9 mg/dL, *p*=0.118). Treatment resulted in 0.5% reduction in A1C vs placebo in the total population. Subpopulation analysis of patients with A1C ≥8.0% at baseline showed that colesevelam HCl lowered A1C by 1.0% compared to placebo (*P*=0.002).
Conclusion: Thus, colesevelam HCl not only lowers LDL-cholesterol, but may also improve glycemic control in patients with T2DM.

	Baseline	Week 12	Δ	LS mean±SE Treatment Effects
A1C (%)				
Colesevelam HCl (n=27)	7.9	7.7	−0.2	−0.5±0.2 *P*=0.007
Placebo (n=32)	8.1	8.3	+0.3	−0.5±0.2 *P*=0.007
Fructosamine (μmol/L)				
Colesevelam HCl (n=27)	299	288	-11	−29±11 *P*=0.011
Placebo (n=32)	318	330	+12	−29±11 *P*=0.011

Supported by Sankyo Pharma Inc.

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HDL evaluation approach for reduction in cardiovascular risk: The HEART audit

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Background and Aims: Type 2 diabetes is associated with a 2–4 fold increase in cardiovascular events and major vascular events can be significantly reduced by appropriate management of dyslipidemia. Treatment targeted towards HDL Cholesterol should be started when primary targets for cholesterol are reached. A 1% reduction in LDL reduces risk of cardiovascular event by 1–1.5% and an increase in HDL by 0.03 mmol/L can reduce cardiac event by 2–4% independent of LDL
The aim of our study was to assess the potential for reducing cardiovascular risk by targeting the secondary lipid target of HDL cholesterol ≥1 mmol/L using Modified release Nicotinic acid, simultaneously assessing its tolerability and impact on glycemic control.
Materials and Methods: 50 patients with type 2 diabetes, with HDL cholesterol less than 1 mmol/L, attending an outpatient clinic in UK, were recruited into the study. Patients on Fenofibrate were excluded. Baseline assessment including BP, HbA1c, lipid profile and UKPDS cardiovascular risk score were calculated at this visit(V1). Eligible patients were then commenced on Modified release Nicotinic acid, starting at 375 mg once daily and gradually increased to 1000 mg once daily over 4 weeks. HDL helpline was available for patients to seek advice during the trial. 34 patients reported for the second visit(V2) in 3 months and were reassessed.
Results: The average age of the patients was 58.2 years, 90% of patients being caucasian in origin. Average duration of diabetes at presentation was 9.5 years. 82% of the patients were already on a statin and 23% of them for hypercholesterolemia. 54% were on Insulin for glycemic control and 22% on thiazolidinediones.
62.5% of the patients showed improvement in HDL over the trial period (range 0.1–0.4 mmol/L). The HDL Cholesterol improved by 12.5% overall from the first to second visit (0.8 vs. 0.9 mmol/L, p<0.05). The improvement in HDL was significantly higher at 18.3% when used as monotherapy. The total cholesterol improved by 15.2% between the two visits (3.8 vs. 3.3 mmol/L) and LDL by 18.8% (1.9 vs. 1.6 mmol/L) whereas triglycerides did not change (2.6 vs. 2.6 mmol/L). The diabetes control had not deteriorated after initiation of this therapy (8.28 vs. 8.17%). The blood pressure remained comparable (135.7/75.8 vs. 134/74.5).
62.5% of the patients showed an improvement in the UKPDS risk score, improving from 23.2 (V1) to 16.2 (V2) (range 0.2–15.5; mean improvement 6.5). The risk score improved by 12.7% overall (23.1 vs. 20.5). Considering the patients who showed improvement in HDL, the risk score improved by 19% (27.9 vs. 22.5).
8 patients (16%) had discontinued therapy during the trial period, 3 due to headache and 2 each due to itching and flushing.

Conclusion: Nicotinic acid has the potential in improving cardiovascular risk in patients with low HDL cholesterol levels. An increase in HDL could be achieved in more than half of the patients initiated on treatment, which would favourably reflect on the total cardiovascular risk. The drug was well tolerated with neutral effects on HbA1c. Randomized trials with larger numbers may be needed to assess clinical outcomes to this approach.

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The safety and efficacy of ezetimibe/simvastatin (E/S) versus atorvastatin (A) in hypercholesterolaemic (HC) patients with type 2 diabetes (T2DM)

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Background and Aims: T2DM is considered a coronary heart disease risk equivalent, requiring aggressive lipid management. This study evaluated the safety and lipid-modifying efficacy of E/S vs A at the usual start doses (E/S 10/20 mg vs A 10 mg and A 20 mg) and alternate start doses (E/S 10/40 mg vs A 40 mg) in HC patients with T2DM.

Material and methods: This was a multicenter, double-blind, randomized, 6-week, parallel-group study. The primary efficacy endpoint was % change from baseline in LDL-C, and secondary efficacy endpoints were % changes from baseline in HDL-C, Total-C, TG, apo B, apo A-I, Non-HDL-C, and hs-CRP, and % attaining LDL-C<100 and<70 mg/dL. Safety was assessed by analyzing adverse experiences (AEs). ANOVA was used for analysis of % change in lipid and non-lipid measures, logistic regression for % attaining LDL-C targets, and Fisher's exact test for safety comparisons.

Results: 1229 patients were randomized from 2300 screened. Baseline patient characteristics were comparable across treatment groups. Efficacy results, shown below, were based on 1198 patients (E/S=480; A=718) included in the modified intent-to-treat population. The incidences of serious drug-related, clinical, laboratory, or prespecified (GI-, gall-bladder-, or hepatitis-related, allergic reaction/rash, ALT, AST, CK) AEs were comparable in the pooled ezetimibe/simvastatin and pooled atorvastatin treatment groups.

Treatment comparison	EZ/Simva 10/20 vs. Atorva 10 mg		EZ/Simva 10/20 vs. Atorva 20 mg		EZ/Simva 10/40 vs. Atorva 40 mg	
Parameter mg/dL	EZ/Simva 20 mg (N=238)	Atorva 10 mg (N=237)	EZ/Simva 20 mg (N=238)	Atorva 20 mg (N=240)	EZ/Simva 40 mg (N=242)	Atorva 40 mg (N=241)
LDL-C						
Baseline mean†	145.0	145.2	145.0	146.6	144.0	145.9
% change from baseline‡	−53.6	−38.3	−53.6	−44.6	−57.6	−50.9
Treatment Difference§# (95% CI)	−15.3*** (−17.7, −12.8)		−9.0*** (−11.5, −6.5)		−6.6*** (−9.1, −4.2)	
HDL-C						
Baseline mean†	44.5	44.8	44.5	46.5	47.4	46.0
% change from baseline‡	8.0	4.3	8.0	4.5	6.3	2.3
Treatment Difference§# (95% CI)	3.6*** (1.5, 5.7)		3.4** (1.3, 5.5)		4.0*** (1.9, 6.0)	
TG						
Baseline median	173.3	191.0	173.3	175.0	175.3	175.5
% change from baseline††	−25.7	−22.7	−25.7	−26.1	−29.3	−28.4
Treatment Difference‡‡# (95% CI)	−4.4* (−7.9, −0.7)		−0.5 (−4.2, 3.0)		−1.1 (−4.7, 2.6)	
hs-CRP (mg/L)						
Baseline median	2.9	2.4	2.9	2.5	2.9	2.6
% change from baseline††	−23.3	−13.7	−23.3	−13.8	−33.3	−28.6
Treatment Difference‡‡# (95% CI)	−10.2* (−18.5, −1.9)		−6.5 (−15.0, 1.4)		−5.4 (−12.5, 1.6)	
Target LDL-C <70 mg/dL n (%)#	142 (59.7)***	51 (21.5)	142 (59.7)***	84 (35.0)	180 (74.4)***	133 (55.2)
Target LDL-C <100 mg/dL n (%)#	215 (90.3)***	166 (70.0)	215 (90.3)**	197 (82.1)	226 (93.4)	214 (88.8)

*p<0.05, **p<0.01, ***p<0.001 for specified between-treatment difference.
†Means; ‡LS Means; §Differences in LS Means; ††Median; ‡‡Median (95% CI)
#Treatment differences are EZ/Simva 10/20 mg minus Atorva 10 mg, EZ/Simva 10/20 mg minus Atorva 20 mg, and EZ/Simva 10/40 mg minus Atorva 40 mg.

Conclusion: Ezetimibe/simvastatin treatment resulted in significantly greater LDL-C-lowering and HDL-C increases at all dose comparisons; generally significantly greater LDL-C goal attainment, particularly at the optional LDL-C<70 mg/dL target; and similar reductions in TG and hs-CRP compared to atorvastatin usual/alternate start doses. Thus, the dual cholesterol-lowering mechanisms of ezetimibe/simvastatin provided additional lipid-modifying benefits over atorvastatin monotherapy in patients with T2DM. Both treatments were generally well-tolerated.
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Efficacy and tolerability of rosuvastatin 40 mg alone or in combination with ezetimibe 10 mg in patients with type 2 diabetes: results from a subgroup of the EXPLORER Study

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Background and Aims: Type 2 diabetes is associated with an increased risk of coronary heart disease (CHD). Lowering LDL-C should be a priority in the control of diabetic dyslipidaemia, yet many patients eligible for statin therapy do not achieve recommended lipid goals in clinical practice. The combination of statins with other lipid-lowering agents is an option for patients who require reductions in LDL-C that cannot be achieved with monotherapy. In EXPLORER, the effect of monotherapy with rosuvastatin (RSV) 40 mg and combination therapy with RSV 40 mg+ezetimibe (EZE) 10 mg for 6 weeks was assessed in patients at high risk of CHD.
Materials and Methods: Patients (n=469) with hypercholesterolaemia (LDL-C ≥160−<250 mg/dL for statin naïve patients; ≥100−<190 mg/dL for statin-treated patients) and a history of CHD, clinical evidence of atherosclerosis or a CHD risk equivalent (diabetes mellitus or a 10-year CHD risk score >20%) were randomised to 6 weeks' treatment with RSV 40 mg alone or in combination with EZE 10 mg. The primary endpoint was achievement of the NCEP ATP III LDL-C goal of <100 mg/dL at week 6. Secondary endpoints included achievement of other lipid goals, and changes in lipids and lipoproteins. In this *post-hoc* analysis, we report on changes in lipid levels and LDL-C goal achievement in patients with or without diabetes from the EXPLORER study, in addition to the overall ITT population. Statistical comparisons were performed on the overall ITT population only.
Results: In the ITT population (n=465), 35.7% of patients (RSV, n=89; RSV+EZE, n=77) had diabetes at baseline. Overall, mean LDL-C levels at baseline were similar in both treatment groups (RSV, 4.93 mmol/L [190.8 mg/dL]; RSV+EZE, 4.89 mmol/L [189.2 mg/dL]). At 6 weeks, LDL-C was reduced to 2.11 and 1.47 mmol/L (81.5 and 56.9 mg/dL) in the RSV and RSV+EZE groups, respectively; significantly greater percentage reductions in LDL-C were achieved with RSV+EZE (Table). RSV+EZE was also associated with significantly greater reductions in TC and TG than RSV (Table). HDL-C levels were increased by both RSV and RSV+EZE (Table). Lipid changes in patients with or without diabetes were consistent with those for the overall ITT population (Table). Achievement of LDL-C goals with RSV and RSV+EZE in patients with or without diabetes was consistent with that observed with the overall population (Table). Both treatments were well tolerated.
Conclusions: RSV 40 mg was effective at improving the atherogenic lipid profile and enabled the majority of patients to achieve LDL-C goals. Combination of RSV 40 mg+EZE 10 mg produced further improvements in lipid levels and enabled additional patients to reach LDL-C goals. The benefits of RSV monotherapy and combination therapy with RSV+EZE in patients with diabetes were consistent with those observed in high - risk patients without diabetes, and in the population as a whole.

	Overall ITT		**With diabetes**		**Without diabetes**	
	RSV N=230	**RSV +EZE N=235**	**RSV N=89**	**RSV +EZE N=77**	**RSV N=141**	**RSV +EZE N=158**
Mean change from baseline (%)						
LDL-C	−57.1	−69.8†	−56.9	−70.5	−57.2	−69.4
TC	−41.5	−51.3†	−41.4	−51.9	−41.5	−51.0
HDL-C	+8.5	+10.8	+6.9	+8.2	+9.5	+12.2
TG	−25.1	−35.4†	−25.3	−35.4	−24.9	−35.5
Patients at goal (%)						
NCEP ATP III LDL-C[a]	79.1	94.0†	75.3	90.9	81.6	95.6
2003 European LDL-C[b]	74.3	93.6†	71.9	89.6	75.9	95.6

[a]LDL-C<100 mg/dL (<2.5 mmol/L)
[b]LCL-C<2.5 or<3.0 mmol/L (<100 or<115 mg/dL), depending on risk category
†p<0.001 vs RSV 40 mg monotherapy

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PS 112

Brain, bone and skin in diabetes

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Evidence of a role for acetyl-L-carnitine in diabetes-related serotonin transmission

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Background and Aims: It has been previously shown that diabetes-associated CNS abnormalities are characterized by

neurotransmission impairments. Several evidences support the concept that a correct mitochondrial function is essential for maintenance of an adequate neurotransmitter secretion. The accumulation of long-chain fatty acids and impaired β-oxidation due to deficiencies in L-carnitine (LC) and/or its esterified derivatives may have deleterious effects on nerve cells function in diabetes by altering plasma or mitochondrial membrane integrity and perturbing intracellular metabolism and energy production. The study has been designed to establish the possible implications of putative LC deficiency and mitochondrial dysfunction as well as potential benefits from acetyl-L-carnitine (ALC) treatment on serotonin (5-HT) transmission in diabetic brain.

Materials and Methods: All studies were carried out after 5 weeks of STZ-induced diabetes (60 mg/kg, i.p.) in rats treated for 14 days with or without ALC (150 mg/kg, i.p.). The LC contents were measured in blood serum and brain by radioenzymatic method. The uptake and the release of [2-^{14}C]serotonin were determined in purified synaptosomes. The mitochondrial membrane potential ($_{\Delta}$Xm) was assayed by spectrofluorimetry using rhodamine123 (Rh123).

Results: Diabetes led to a pronounced decrease in LC levels of serum (46.4±3.2%) and brain (31.7±2.7%) vs control, $p<0.05$. Diabetic mitochondria showed elevation of Rh123 fluorescence (21.0±0.19%, $p<0.01$) vs control, indicating decreased $_{\Delta}$Xm. These changes were associated with the decline in both synaptosomal ATP and respiratory control ratio (ATP/ADP), respectively 2.15±0.09 vs 3.19± 0.11 nmol/mg protein and 4.23±0.21 vs 6.85±0.38, $p<0.05$. Compared to control, diabetes caused 40.3±3.7% elevation of spontaneous release and 24±2.9% decrease in uptake of 5-HT in synaptic endings, that was accompanied by 24.3± 1.8% downregulation of synaptosomal Na^+,K^+-ATPase activity, $p<0.01$. 5-HT release was 2-fold more profound in diabetes vs control upon *in vitro* stimulation by 30 mmol/l KCl, suggesting augmented diabetes-induced quantal mode of neurotransmitter secretion. ALC treatment normalized serum and brain LC levels and diminished the development of cellular respiration deficits by 81.0±5.7% restoration of $_{\Delta}$Xm with corresponding, respectively 72.5± 4.3% and 78.3±5.6%, recovery of ATP content and ATP/ADP, $p<0.05$. It is most likely that attenuation of mitochondrial dysfunction following ALC administration contributed to significant currently established improvement of Na/K pump activity, 5HT uptake and spontaneous release except for the increased synaptosomal response to KCl, which was affected only slightly. It is noteworthy, that KCl-induced 5-HT release is largely independent on ATP synthesis, since this secretogue directly affects plasma membrane potential and ion fluxes. ALC treatment had no lowering effect on blood glucose and brain sorbitol.

Conclusion: The data provide evidence that under energy-depleted conditions related to diabetes the decrease in Na^+ and K^+ gradients and subsequent reversal of 5-HT transporters in synaptosomes may be the key mechanism underlying reduced uptake and enhanced spontaneous release of 5HT. ALC modulatory action on serotoninergic system is dependent on energy metabolism, rather than being the consequence of a direct effect on the neurotransmitter secretory machinery.

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Lutein effect in the retina and hippocampus of diabetic mice

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Background and Aims: It has been repeatedly suggested that oxidative stress may play an important role in the pathogenesis of diabetes complications, and it is clear that the elevated glucose levels seen in diabetes and the existence of oxidative stress are inseparable.

The diabetes-induced production of reactive oxygen species (ROS) activates the nuclear factor kB (NFkB), which activates a variety of target genes which are linked to the development of diabetic complications.

Among these target genes are include the ones involved in inducing programmed cell death, because although in general, this nuclear factor is considered antiapoptotic, it can be proapoptotic depending of the cell type and the stimuli.

Diabetic retinopathy is a frequent complication of diabetes, and in the recent years, evidence is emerging that diabetes affects also the Central Nervous System by impairing the functional and structural integrity of the brain in diabetic patients. One of the mechanisms by which hyperglycemia is known to cause neural degeneration is via the increased oxidative stress that accompanies diabetes.

The aim of this study was to confirm the early biochemical and functional changes in retina and hippocampus of diabetic mice. Oxidative stress markers, NFkB activity and functional tests for retina and hippocampus were studied. We also tested the effects of a treatment with lutein (a natural antioxidant without hypoglycemiant properties),compared to insulin therapy.

Materials and Methods: Male albino mice were induced diabetic by alloxan injection and divided into subgroups: control, control+lutein, diabetic, diabetic+lutein, diabetic+ insulin and diabetic+insulin+lutein. Treatments started on day 4 after alloxan injection and animals were sacrificed on day 14. Malondialdehyde (MDA) and glutathione (GSH) concentrations, as well as Glutathione peroxidase (GPx) activity were measured as oxidative stress markers. Functional tests

for retina and hippocampus were performed: electroretinogram and Morris water maze test (a test for evaluating spatial memory). NFκB activity was measured in transgenic mice containing the NFkB-luciferase reporter gene.
Results: After 15 days of diabetes, lipid peroxidation (MDA) was increased in the retina and hippocampus, while the antioxidants defences (GSH and GPx) decreased. NFkB activity was increased in both tissues. We observed impairment of the electrorretinogram in diabetic mice, but no differences were observed when examined for spatial learning ability. However, a positive correlation was found between latencies in the Morris water maze test and HbA1c levels. Lutein treatment (even under hyperglycaemic conditions), as well as insulin therapy, prevented all these alterations. Retina appears to be affected earlier than hippocampus by diabetes-induced oxidative stress.
Conclusion: Although a proper glycemic control is desirable to prevent the development of diabetic complications, it is not always achivable. Lutein, a natural antioxidant which is able to modulate NFkB activity, and a scavenger of peroxynitrites, could be an appropriate coadyuvant treatment for the alterations observed in this study. Further studies are needed to elucidate the mechanisms that make the retina more susceptible to diabetes than hippocampus at early stages of these disease, and to determine the role of NFkB on these mechanisms.
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Model for investigating type 2 diabetic patients with suspected sleep apnoea

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Background and Aims: The prevalence of sleep apnoea has been reported to be increased in type 2-diabetic patients. It seems to be associated with an additional cardiovascular risk. Full polysomnography is time consuming and expensive. An approach based on a logistic regression model and an ambulatory monitoring for cardiorespiratory abnormalities as well as clinical features and signs and symptoms of diabetic complications has been developped to determine which diabetic patient needs full polysomnography.
Materials and Methods: The variables entered in the regression model were derived from 60 type 2 -diabetic patients of both sexes (mean age 60 years, BMI 30.9 kg/m^2, mean diabetes duration 8 years, mean HbA1c 8.5%) referred to the outpatient clinic for tighter diabetes control. Besides autonomic function tests to assess cardiovascular autonomic neuropathy, motor and sensory nerve conduction velocities of the tibial and sural nerves, as well as an ambulatory monitoring for cardiorespiratory abnormalities recording airflow, respiratory movements, arterial oxygen saturation, heart rate and body position were done.
Results: The prevalence of sleep apnoea was 20% in the population studied. The logistic regression analysis showed that the age, the motor nerve conduction velocity, and a cardiovascular autonomic neuropathy are predictors of the sleep apnoea. Neither the BMI, nor the duration of the disease, other diabetic complications, or the HbA1c value are associated with sleep disturbances. A cut off probability of 0.30 gave the prediction model a sensitivity of 75% and a specificity of 89% to predict a RDI of > 30.
Conclusion: The patients age, the motor nerve conduction velocity and a cardiovascular autonomic neuropathy are useful predictors of a sleep apnoea in type 2- diabetes.

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Obstructive Sleep Apnoea (OSA) is independently associated with increased prevalence of the metabolic syndrome but not insulin resistance status

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Background and Aims: Epidemiological evidence suggests a link between OSA and components of the metabolic syndrome (MS) but whether OSA is associated with the overall syndrome independent of obesity remains unclear. Furthermore, the agreement between MS and insulin resistant state when applied to patients with OSA has not been previously determined.
Materials and Methods: We prospectively recruited 41 subjects with OSA and 38 controls. Anthropometric measurements, lifestyle questionnaires, glucose, lipids, insulin and blood pressure (BP) were measured after an overnight fast. Insulin resistance state was defined as homeostasis model assessment (HOMA) value >3.99 and MS were diagnosed according to the International Diabetes Federation (IDF) criteria.
Results: Subjects with OSA were more obese, more insulin resistant, more hyperglycaemic, had higher epworth score (measure of day time somnolence) and systolic blood pressure levels. The prevalence of MS was higher in OSA compared with non-OSA subjects (74% vs 24%, $p<0.001$). The prevalence of microalbuminuria in both groups was

negligible. Regression analysis model, adjusting for age, BMI and smoking showed that the prevalence of MS was 5.9 (95% CI 2.0–17.6) times higher in subjects with OSA. Triglyceride (p=0.031), glucose (0.023) and Epworth scores (0.003) vales are independently associated with OSA after adjusting for BMI and other covariates whilst insulin resistant status was found not to be significant. Using ROC curve analysis, we found that a waist circumference of > 105 cm and a neck circumference >40 cm would predict MS in patients with OSA at 75% sensitivity and 65% specificity. Using κ statistics, we found that the agreement between MS and insulin resistant status in this cohort is poor.
Conclusion: Thus, OSA is shown to be associated with the MS independent of obesity. This independent association is driven mainly by the increased triglyceride, glucose and Epworth score values but not insulin resistant status. This observation suggests an alternative pathogenic factor which mediates the increased cardiovascular risk in patients with OSA and MS, other than that due to insulin resistant state. The independent link between Epworth score and MS in patients with OSA implicates the role of daytime sleepiness and chronic hypoxia as a potential mediator.

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The components of metabolic syndrome: their impact on the extension of atherosclerosis assessed by the carotid intima-media thickness

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Background and Aims: The carotid intima-media thickness (IMT) determined with high resolution ultrasound is an accepted parameter for the assessment of early atherosclerosis.The presence of metabolic syndrome (MetS) represent a risk for the development of both type 2 diabetes and cardiovascular diseases. The atherogenic impact of different components within the MetS is not well established. There is no convergent information on how some MetS component combinations (different numbers and types) can influence preclinical atherosclerosis assessed by carotid IMT. The purpose of this study was the assessment of atherogenic potential of different components of the MetS, using carotid IMT like early atherosclerosis marker.
Materials and Methods: We included 131 patients who fulfill the IDF 2005 criteria for the diagnosis of the MetS, men and postmenopausal women, non-smokers, non-diabetic, without a previous treatment for dyslipidaemia. Carotid IMT was considered to be the maximum value measured bilaterally from multiple incidences. The studied groups do not differ in term of age and gender distribution.
Results: IMT increased gradually with the number of MetS components (without counting increased waist circumference, mandatory for the diagnosis) present at the same patient (**3 components:** 0.96+/−0.18 mm vs **2 components**:0.91+/−0.15 mm, p=0.04 and **4 components**:1.05+/−0.17 mm vs **3 components:** 0.96+/−0.18 mm, p=0.04). Low HDL-cholesterol levels and the presence of prediabetes showed the strongest impact on the carotid IMT, for the patients with **2 components** of MetS. For the patients who had **3 components** of the MetS, the low HDL-cholesterol/high triglycerides/hypertension combination was more atherogenic than prediabetes/high triglycerides/hypertension combination (1.02+/− 0.18 mm vs 0.92+/− 0.17 mm, p=0.03).
Conclusion: An increase in the number of components of the metabolic syndrome was associated with an increase in the mean carotid intima-media thickness. Among the components of the metabolic syndrome, low HDL-cholesterol and prediabetes showed a stronger impact on carotid atherosclerosis.

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Gender risk of non-fatal stroke in type 2 diabetic patients differs depending on the type of treatment

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Background and Aims: The significant decrease of the gender differences in cardio-vascular risk among type 2 diabetics (T2D) were detected in a number of epidemiological studies. The reason of this phenomenon remains unknown. The different types of treatment are used to treat T2D, including insulin, the excess of which was shown to result in hyperandrogenization of the T2D women (Poretsky L.1991). However, the stratification by type of treatment at an estimation of gender risk in T2D was not carried out till now. The aim of present observational cross-sectional study was the estimation of the gender-related risk of non-fatal stroke in T2D depending on the type of antidiabetic treatment in Ukraine.
Materials and Methods: In Dec 2005, the data from 11 Ukrainian regions for all living type 2 diabetics already included into the nationwide population-based diabetes register (start of creation Jan 2001) was extracted. Male/female relative risks (RRs) for non-fatal stroke prevalence were calculated in 3 treatment groups: Diet only (DO)

7273/15901; oral antidiabetic drugs (OAD) 15109/33913; insulin: 5529/12462 male/female respectively. 95% confidence intervals (95 CI) were estimated by logarithmic transformation. Chi-square test was used to compare the differences between groups. Male/female odds ratios (OR) were estimated using a logistic regression model after adjustment for age and diabetes duration. The study protocol was approved by the ethics committee of the Institute of endocrinology and metabolism, Kiev.

Results: The higher prevalence of stroke among men was in those patients treated with OAD or DO: RRs=1.38 (1.26–1.51) and 1.48 (1.25–1.76), respectively. The gender differences were absent among those treated with insulin: RR=0.98 (0.87–1.10), which is significantly lower ($p<0.001$) than corresponding OAD or DO RR's levels. Men's stroke OR's were significant ($p<0.001$) for DO and OAD treated patients: 1.54 (1.29–1.84) and 1.45 (1.31–1.59) respectively, but not for insulin-treated T2D: 1.06 (0.96–1.16), $p=0.22$.

Conclusion: The gender risks of not-fatal stroke in T2D are significantly different depending on the type of treatment. Further research could clarify if it depends upon insulin-related hyperandrogenisation in T2D women.

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Impact of diabetes on type I collagen in relation to availability of vitamins. Role of nicotinamide and nicotinoyl-GABA treatments

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Background and Aims: Diabetes-related dermal changes, impaired wound healing, osteopenia and decreased bone strength may be associated with altered metabolism of type I collagens (I-C). It is assumed that alterations in I-C amino acids as a result of gene polymorphism may strongly affect protein properties and physiological function; however, very limited evidences are present at the moment regarding amino acid (AA) composition of bone and skin I-C and its relevance to abnormal availability of vitamins which are necessary for collagen synthesis in diabetes. We tested whether nicotinamide (NAm) and nicotinoyl-GABA (N-GABA) as the drugs with a wide range of metabolic effects can influence I-C formation and its AA composition as well as vitamins availability in diabetes.

Materials and Methods: After 4 weeks of STZ-induced diabetes (60 mg/kg, i.p.), male Wistar rats were injected for 14 days with or without NAm or N-GABA (200 mg/kg, i.p.). The contents of I-C after its acid extraction from bones or skins with following step-wise salting out were estimated by the amounts of hydroxyproline (Hyp). The amino acids were assayed by cation exchange chromatography.

Results: Vit. C, PP and E (α-tocopherol) contents were 19.1± 6.4, 31.0±1.9, 21.8±2.0 vs 43.9±7.1, 54.3±3.8, 14.6± 2.4 in blood serum and 601.3±72.5, 540±37, 49.0±7.2 vs 1893± 279, 795±52, 31.0±5.1 in liver, respectively in diabetes vs control (nmol/g tissue, $p<0.05$). Impaired availability of the vitamins may be ascribed to their increased consumption by elevated oxidative stress which we previously identified in various tissues from diabetic rats and may also be due to reduced NADPH-dependent regeneration of vit. C since its level was reversed towards normalisation after NAm and N-GABA treatment, $p<0.05$. Restoration of circulatory and liver vit. PP contents and slight decrease in vit. E levels were also seen due to both treatments. I-C depositions in bones and skins were lower in diabetes vs control as it is evident from respectively 5.2- and 2.4-fold decrease in Hyp contents, $p<0.01$. Diabetes-associated changes in amino acid composition of I-C were largely independent on its localization mainly affecting those AA which are known to be involved in helix formation, cross-linking and charging of the molecules. Besides Hyp lowering, diabetes also significantly reduced collagen Gly, Ala contents in skins and additionally hydroxylysine and Pro in bones, whereas collagen Lys, His, Arg, Glu, Thr, Leu, Phe contents in both organs and Asp, Ser and Tyr in skins were elevated, $p<0.05$. NAm and N-GABA were able to partially restore I-C depositions and normalise AA contents with the efficacy greater in case of N-GABA treatment. The diabetes-induced alterations could be related to abnormal hepatic function as evidenced by 1.7- and 1.3-fold increase in the total amount of free amino acids in liver and serum and Fischer index (Leu+ILe+Val/Tyr+Phe) raised in serum (3.58±0.41 vs 1.73±0.16 in control, $p<0.05$). Drug treatments exhibited similar beneficial effects on liver and serum AA.

Conclusion: The study confirms the relationship between the diabetes-associated decrease in I-C content, alterations of its amino acid composition and impaired availability of vitamins. The corrective effects of NAm and N-GABA on collagen synthesis and processing, additionally with other possible contributing factors, may be attributed to preservation of vitamin C and E functions.

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Longitudinal changes in bone mineral density in pre- and postmenopausal women with type 1 and type 2 diabetes

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Background and Aims: The aim of this longitudinal study was to investigate the spontaneous changes in bone mineral density (BMD) in pre- and postmenopausal women with type 1 (T1D) and type 2 (T2D) diabetes mellitus.
Materials and Methods: 51 pre- and postmenopausal women with type 1 or type 2 diabetes mellitus from the out patient clinic at the Steno Diabetes Center were examined. The population was divided into four groups premenopausal type 1 (n=19), postmenopausal type 1 (n=15), premenopausal type 2 (n=7), and postmenopausal type 2 (n=10)). At two occasions three years apart BMD in the total body, lumbar spine, femoral neck and nondominant forearm were determined.
Results: In the group of postmenopausal type 1 diabetics a significant decrease in BMD could be detected (total body BMD −0.87% p.a). The change in BMD correlated significantly and positively with duration of the menopause suggesting a large fall around the menopause. There were no statistically significant changes in BMD in any of the sites measured in premenopausal T1 or T2 diabetics or in postmenopausal T2D.
Conclusion: BMD decreased in postmenopausal type 1 diabetics, but not in premenopausal T1D or T2D. The decrease in postmenopausal T1D were not larger than what could be expected in the normal healthy population.

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The effect of diabetes mellitus type 2 on the bone mineral density of males and postmenopausal females

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Background and Aims: The majority of previous studies have demonstrated a favorable effect of diabetes mellitus type 2 on bone mineral density (BMD). It is still unclear whether the degree of such effect is similar for the two genders. The purpose of the study was the comparison of the degree by which diabetes mellitus type 2 affects the BMD of both genders.
Materials and Methods: One hundred and forty one diabetic males [DM, age 56.9±5.8 years (mean value±1 SD), BMI 26.8±3.1 kg/m^2, disease duration 8.2±3.4 years], 137 diabetic postmenopausal females (DW, 57.3±4.1, 26.7±3.5, 7.4±3.3), 60 healthy males (HM, age 56.1±6.7, BMI 26.8±3) and 301 healthy postmenopausal females (HW, 56.9±3.3, 26.2±3.6) were studied. BMD was measured at the L2-L4 vertebrae and the femoral neck (FN) by DXA. None of the subjects suffered from any other disease or had received any medication with a known effect on bone metabolism. They were all matched for age and BMI. None of the diabetic subjects received insulin. The sex- and age- adjusted BMD values (Z-scores) were used to compare the diabetes' effect between the 2 genders.
Results: Regarding the vertebral BMD values, diabetic persons of both genders presented significantly higher BMD values compared to their respective healthy ones ($p<0.001$). On the other hand, although in both genders diabetic patients presented higher FN BMD values compared to healthy ones, only between DW and HW this difference was significant ($p<0.001$). Both DM and HM presented significantly higher vertebral Z-score values compared to their female counterparts ($p<0.001$). Regarding the FN, only HM presented significantly higher Z-score values compared to HW ($p<0.05$), while DM and DW did not differ significantly. The proportions of osteopenic-osteoporotic subjects (WHO criteria) were significantly higher in the healthy compared to their diabetic counterparts in both genders ($p<0.01$). No significant differences were observed in the same proportions between diabetic males and females. In all groups, but HM, a significant positive correlation was observed between either vertebral or FN BMD values and BMI ($r=0.19–0.30$, $p<0.05$). No correlations were observed between the BMD values and the disease duration or the HbA1c concentrations in diabetic subjects.
Conclusion: Diabetes mellitus type 2 seems to exert a favorable effect on the bone mineral density of diabetic patients of both genders. This effect seems to be stronger in the trabecular bone of diabetic males and is irrespective of the body weight. Rather the presence than the duration of diabetes seems to correlate to the disease's effect on bone mass.

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Detection of free elastin derived peptides among diabetic children

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Background and Aims: Elastin breakdown products are found in the serum of all human subjects. The presence of these elastin-derived peptides (EDP) and the corresponding antibodies in circulation leads to formation of circulating immune complexes (CIC). The aim of this study was to

determine if serum level of free-EDP (unbound in CIC) correlate with the development of vascular complications in diabetic children.

Materials and Methods: To this end we used a method for detecting immune complexes CIF-ELISA. Isolation of a new glycoprotein complement inhibition factor (CIF) from the parasitic plant *Cuscuta europea* seed, which appears to bind specifically to complement component C3 has been provided an unique tool for the measurement of immune complexes by means of ELISA-type techniques (CIF-ELISA). Free EDP were detected by using a two-step method consisting of: (i) CIF-ELISA to remove immune complexes followed by (ii) an elastin-specific ELISA for detection of EDP.

The levels of free EDP were studied in sera of 81 diabetic children (mean age-13.46±3.51 years, diabetes duration 5.17±4.21 years). Forty-two of the children had vascular complications (group 1) and 39 were without vascular complications (group 2).

Results: Twenty one healthy children (mean age-12.6± 2.47 years) were used as controls. Diabetics showed statistically significant higher levels of free EDP (68.1±25 vs. 51± 12.5 ng/ml; p=0.003) compared to the control group. In group 1, free EDP showed statistically significant higher levels than controls (78.9±25.6 vs. 51±12.5 ng/ml; p=0.0001). Thirty eight of 81 (47%) patients were positive for free EDP (30/42–71% in group 1 and 8/39–21% in group 2). Free EDP levels in all diabetics showed a correlation with insulin dose (r=0.23; p=0.041), retinopathy (r=−0.20; p=0.06), and microalbuminuria (r=0.44; p=0.0001). Patients who had vascular pathology showed a correlation of free EDP with microalbuminuria (r=041; p=0.01), retinopathy (r=0.32; p=0.041), insulin dose (r=0.37; p=0.02), HbA1c (r=0.35; p=0.03), systolic blood pressure (r=0.30; p=0.045) and total cholesterol (r=0.36; p=0.02).

Conclusion: These findings suggest that elevated levels of free EDP are associated with the development of diabetic vascular complications in children.